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Title of Dissertation: "A study on the bionomics of *Anopheles darlingi* Root (Diptera: Culicidae) in Belize, Central America"

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ABSTRACT

A Study on the Bionomics of *Anopheles darlingi* Root (Diptera: Culicidae)
in Belize, Central America

By

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Uniformed Services University of the Health Sciences, 2004

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Interdisciplinary studies were conducted to describe the bionomics of the malaria vector *Anopheles darlingi* Root in Belize, Central America. Studies investigated the following: nightly adult biting patterns; seasonal population densities; flight behavior patterns; the role of overhanging bamboo in larval habitat preference; the association between deforestation and bamboo growth; and the associations between land cover and river characteristics to the distribution of positive larval habitats.

Results from all-night biting studies show *An. darlingi* to exhibit a bimodal peak activity pattern with biting continuing throughout the night at similar rates both indoors and outside of an experimental hut ($I:O=1.00:0.96$). Population studies show *An. darlingi* to have its densest populations during seasonal transitional months including January and May/July. Results from flight behavior studies of *An. darlingi* females, using a newly designed portable hut, show the highest recapture rate was made at the 0 M distance (28.9%) from a fixed release point, then declined from 11.6% at 400 M to 5.8% at 800 M.

Report Documentation Page			Form Approved OMB No. 0704-0188		
<p>Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p>					
1. REPORT DATE MAR 2004	2. REPORT TYPE	3. DATES COVERED 00-03-2004 to 00-03-2004			
4. TITLE AND SUBTITLE A study on the bionomics of Anopheles darlingi Root (Diptera:Culicidae) in Belize, Central America			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Uniformed Services University of the Health Sciences,F. Edward Hebert School of Medicine,4301 Jones Bridge Road,Bethesda,MD,20814-4799			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES The original document contains color images.					
14. ABSTRACT see report					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF: a. REPORT b. ABSTRACT c. THIS PAGE unclassified unclassified unclassified			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 320	19a. NAME OF RESPONSIBLE PERSON

Habitat preference studies indicate that overhanging bamboo is not an *An. darlingi* breeding site selection criterion. Experimental plots in which bamboo was hung above detritus consistently showed significantly fewer *An. darlingi* larvae than plots with detritus alone and similar numbers as in open water control plots. Studies combining field mapping with remote sensing along “cleared” (i.e., deforested) and “undisturbed” (i.e., forested) transects within two river systems showed no associations between land cover adjacent to the rivers and bamboo growth. Results were consistent using both SPOT (20-m resolution) and IKONOS (4-m resolution) satellite imagery.

In addition, overhanging bamboo was not the primary contributor to the formation of potential *An. darlingi* larval habitats formed within a 48-km transect of the Sibun River. Instead, components of trees (i.e., fallen trunks, etc.) were found to be the predominant landscape feature associated with habitat creation. Using IKONOS imagery, no associations were found between the locations of positive habitats and land cover or river characteristics. However, the average distance from detritus mats containing *An. darlingi* larvae to houses located within a 1,000-m search radius was significantly less than the distance from negative habitats.

KEY WORDS (Indexing): *Anopheles darlingi*, biting patterns, seasonal densities, flight behavior, bamboo, remote sensing and Belize.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES

**A study on the bionomics of *Anopheles darlingi* Root (Diptera:Culicidae) in Belize,
Central America**

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DEPARTMENT OF PREVENTIVE MEDICINE AND BIOMETRICS
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

By

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March 2004

ACKNOWLEDGEMENTS

I would like to thank all of the members of my doctoral committee for their guidance in writing this dissertation: Dr. Donald R. Roberts (Major Dissertation Advisor); Dr. Richard G. Andre (Committee Chairperson); Dr. John Cross; Dr. Susan Langreth and Mrs. Penny Masuoka. Gratitude is extended to Dr. Eliska Rejmankova, Department of Environmental Science & Policy, University of California Davis for her full financial (NIH Grant #R01 AI49726 entitled: "Ecological Determinants of Malaria in Belize") and logistical support of my research activities necessary to complete the following studies. Special appreciation goes to Dr. Roy Vogtsberger of Hardin-Simmons University in Abilene, Texas for sacrificing his time, gratis, to help in the identification of aquatic invertebrates collected during the Sibun River survey.

I would also like to recognize those people of Belize who, without their approval and assistance, the success of this research would not have been possible: Dr. Errol Vanzie, Director of Health Services, Ministry of Health (MOH); Dr. Jorge Polanco, Deputy Director of Health Services (MOH); Mr. Derric Chan, Belize Riverkeeper Program; Ms. Natalia Andrews, National Meteorological Service; Dr. Ed Boles, University of Belize; Mr. Julio Reyes, University of Belize; Mr. Emerson Garcia, University of Belize; Mr. Rigeberto, Sibun Watershed Association; Mr. Jorgen Rahm, Belize Bamboo Project; the chairmen of both San Roman and Armenia villages.

Special appreciation goes to Mr. and Mrs. Ramon Galvez for the gracious use of their land and wonderful hospitality. I would also like to express special appreciation to both Mr. Ireneo Briceno and Mr. Russell King for their tireless efforts in helping me conduct these studies while always maintaining a great sense of humor and camaraderie.

Finally I would like to thank Dr. John Grieco for extending both his professional and personal companionship during this endeavor.

DEDICATION

This dissertation is dedicated to family and friends for their unendless support and patience over the years required to complete this research.

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Chapter 1

General Introduction

GENERAL INTRODUCTION

Today, approximately 40% of the world's population, mostly those living in the world's poorest countries, is at risk of malaria (WHO 2003). The disease was once more widespread, but it was successfully eliminated from many countries with temperate climates during the mid-20th century. Malaria is found throughout the tropical and subtropical regions of the world and causes more than 300 million acute illnesses and at least one million deaths annually.

Malaria transmission has increased in recent years due in part to less use of effective insecticides, parasite drug resistance and the movement of infected and/or susceptible human populations throughout endemic areas. In addition, population growth has led to an increase in land use and land cover changes as a result of expanding human settlements, agricultural growth and deforestation for logging. Such landscape changes alter anopheline vector breeding habitats potentially leading to variations in disease transmission dynamics (Walsh et al. 1993; Patz et al. 2000; Conn et al. 2002).

Thus, it is vital that malaria epidemiological studies continue in endemic countries in order to provide sustainable prevention and control solutions. Historically, control measures that have been directed at the vector population have shown the greatest success (Russell et al. 1963). This demands a thorough understanding of the bionomics of individual local anophelines (Tonn 1983; Zimmerman 1992; Collins and Paskewitz 1995) and the development of applicable tools to predict high-risk transmission areas (Andre et al. 1994; Roberts and Rodriguez 1994; Clarke et al. 1996). Information from these studies can then be used in decision-making processes necessary for surveillance and resource allocation within individual malaria control programs. Because each area is unique in its

vector population and because characteristics of these populations are so varied, a control strategy must be developed to deal with the specific needs and lifestyles for that region (WHO 1995).

Demographics of Belize

The country of Belize is located in Central America and is bordered by Mexico to the north, Guatemala to the west and south and the Caribbean Sea to the east (Figure 1). Belize has six political districts with an estimated geographic area of 22,963 km² of which 95% is located on the mainland and 5% is distributed over more than 1,060 islands. The capital, Belmopan, is located in the Central Cayo District. The last full population census took place in 2000 when 248,916 inhabitants were counted. However, inter-censal Labor Force Surveys are conducted annually to estimate mid-year population size and by 2001, the population of Belize stood at 249,800; 52% rural and 48% urban (Central Statistical Office). This is believed to be a direct consequence of immigrants settling in the countryside. The majority of the population lives in the Belize District (68,197). The southernmost Toledo District has the lowest population (23,297) and the central Cayo District is the fastest growing district with a 29.3% (14,871) increase between 1991 and 2000.

The demographics of Belize have remained fairly constant with 41% of the population below 15 years of age, less than 5% over 65 years old, and an average life expectancy of 74 in the 1990's (Figure 2). Countrywide, although the overall ratio of male to female is almost 50:50, there is a 5% difference in favor of women in the 15-44 age group and a 15% difference in the 20-24 age group.

Some 14% of the population represents immigrants from neighboring Central American countries, contributing to the population growth rate of 2.8% per annum since 1991 and representing 76% of the total foreign-born population. The country of Guatemala has remained the single biggest contributor accounting for 42.5% of the foreign-born population. The second biggest group is from El Salvador (17.6%) with Honduras representing 14%, an increase from 9% in the 1991 census. The majority (60%) of foreign-born persons has settled in the rural areas and represents 16% of the total rural population. The flow of foreign-born persons during the nineties (16,366) was higher than in the 1980's (12,726).

Belize is an ethnically diverse country with Mestizo (48.7%) representing the largest ethnic group followed by the Creole (24.9%). Other ethnic groups include Garifuna, East Indian and Mennonite (Figure 3). At the district level, Mestizos were the largest ethnic group in the districts of Corozal (76%), Orange Walk (77%), Cayo (64%) and were only slightly less (30 % vs. 31%) than the Garifuna group in Stann Creek. Creoles are the largest group in the Belize District (59%), and in Toledo the Mayas (65%) outnumber all other ethnic groups. All districts also have Mennonite communities forming a unique German-speaking population. The Roman Catholic denomination represents the religion with the largest number of followers in the country comprising 49.6% of the total population (Figure 4).

In the year 2000, approximately 20% of persons greater than two years of age reported no formal level of education although an overall literacy rate of 70% was reported countrywide (Central Statistical Office, 2000). There is noticeable urban/rural difference in regards to education with 17.4% of the urban population having reached

secondary level while only 7.4% of the rural population having done so. Differences at district levels occur as well. The Belize District had 18.7% of its population reach secondary level in comparison to only 6.4% in the Toledo District. For the other four districts, between 8-11% of their populations reached this level. These figures may reflect that more job opportunities are available in the urban areas (Belize District), attracting the more educated rather than the educational opportunities between districts being unequal.

Fertility analyses in the year 2000 indicate that women of reproductive age reported having an average of 3 children. The average number of children per woman is higher in rural areas (3.4) than in urban areas (2.5). Creole women, on average, have fewer children and Maya women more children than women in other major ethnic groups. The 2000 census showed that women with no education had an average of 4 children, while women who completed high school had an average 1.2 children.

Approximately one quarter of the employed population is engaged in agriculture and forestry activity. Wholesale and retail trade is the second biggest industry accounting for 16% of the employed population followed by general government services (13%), construction (9%) and tourism (9%). The main industry in the urban areas is wholesale and retail sales, while agriculture is the main industry in the rural areas. The agricultural industry was strongest in Toledo, Corozal and Stann Creek Districts. Fishing and fish processing is strongest in Stann Creek District, while tourism is strongest in the Belize (12%) and Cayo (10%) Districts. The mean income is \$835.00 per month with males earning more on average than females. The Toledo District reported the highest percentage (23%) of people that earned less than \$1,400.00 per annum, while the Belize District reported less than 1% with an annual income less than this amount. The Belize

District also reported the highest percentage (3%) that earned more than \$34,560.00 per annum.

Ecology of Belize

The country of Belize can be divided into two generalized ecoregions (Rubio-Palis and Zimmerman 1997) between the northern and central/southern portions of the country. The northern districts of Corozal and Orange Walk comprise coastal savannah characterized by scrub bush and marshland. Sugarcane is the main cash crop in this region, but vegetable and citrus plantations also can be found within Mennonite communities. The central/southern districts of Belize, Cayo, Stann Creek and Toledo are defined as piedmont interior lowland forest characterized by the growth of secondary forests and citrus plantations including orange and banana. A mountain range, the Maya Mountains, occurs in the central interior region of the country bordering Guatemala to the west with an average elevation of approximately 850 m and a peak elevation of 1,124 m (Victoria's Peak). Offshore, the Belize Barrier Reef is the second largest in the world and the largest in the Western Hemisphere.

Belize's climate is defined as sub-tropical with an average temperature of 26.1°C and a range of 10°C to 35°C (National Meteorological Service). The focal months of the dry season throughout the country are February, March and April and a distinct wet season exists from June to November, although most locations experience a drier period in August. Between the wet and dry seasons (i.e., November-February) there exists a cool transitional period. The coolest temperatures, averaging 23.8°C, exist during the wet season, and the highest temperatures, averaging 27.2°C, occur during the dry season. The northern districts experience an average annual rainfall of 1,500 mm (60 inches),

which is much less than the average 3,800 mm (150 inches) that falls in the southern regions. Some 60% of annual precipitation occurs during the rainy season and is produced primarily by tropical systems including tropical hurricanes. The rainy season in the southern districts usually begins earlier (i.e., middle of May) than in the northern districts.

Land Use and Deforestation in Belize

As of 1994, the most recent report, the majority (79%) of Belize remained under natural vegetation cover (Fairweather and Gray 1994). Although almost a decade prior to the beginning of the present research, the trends in land use remain the same. Approximately 1.4 million hectares (65%) of the natural vegetation comprised high broadleaf forest. Other types of vegetation cover included coastal mangrove formations (1.0%), thickets (3.9%), closed and open pine forest (2.9%) and savannah (8%). The remaining land area of Belize (approx. 688,800 ha) was dominated by agriculture. Most of this land was under Mennonite ownership and is used for corn, sorghum and bean crops as well as pastureland. Approximately 24,300 ha are devoted to sugar cane production, primarily in the northern districts of Corozal and Orange Walk. An additional 11,300 ha are planted in citrus orchards in the Stann Creek Valley, of the central Stann Creek and Cayo districts, while banana plantations occupy 2,400 ha in both the Stann Creek and southern Toledo District.

In 1993, protected areas covered 33.4% (767,000 ha) of the country (Forest Department 1993). Of this area, 38% is managed for biodiversity conservation, education and research purposes. The remainder is within forest reserves allowing extractive use. However, 50% of these forest reserves are treated as protected forest due

to slope or soil moisture constraints. The area outside the protected area comprises government and privately owned lands that are used for eco-tourism ventures and extraction of both selective timber and non-timber forest products

Among the environmental issues facing Belize are deforestation and the management of forest resources. Deforestation as defined in this study is synonymous with land clearing (Ledec 1992). The rate of land clearing for agriculture increased from 8,996 ha per year in the late 1980's to about 24,989 ha in the late 1990's. It is estimated that 6,070-12,140 ha of closed forest are cleared annually for agriculture. From 1989/92 to 1994, there was a loss in forest cover of approximately 78,100 ha throughout mainland Belize (White et al. 1996). More than 90% occurred on private and national land outside of protected areas, but forest cover loss in protected areas (i.e., primarily National Forest Reserves) accounted for about 6,680 ha or 8.8% of the total loss. This indicates an inadequacy of resources to implement policy. In addition, several locations along riverbanks exhibit illegal land clearance for citrus production within the 66-foot buffer zone established by the Forestry Department and, surprisingly, class 4-land (recommended for forest management and plantation) is being used for agriculture. If this trend does not reverse, only steep and otherwise unsuitable land will be under forest cover within the next 50-100 years. However, it is generally believed that the amount of deforestation that has occurred in Belize is much less than has occurred in other rain forest countries (Forestry Department 1993).

Examination of the mainland shows the most extensive forest losses between 1989/92 and 1994 occurred in the Cayo and Toledo Districts, where 20,090 ha and 19,035 ha, respectively, were cleared (Figure 5). Deforestation (1.5%) and soil erosion

(1.0%) comprised small proportions of overall environmental concerns to the general public during the 2000 census but increased to 4.0% and 3.2%, respectively, at the district level for both Cayo and Toledo Districts where the majority of land clearing is taking place (Central Statistical Office 2000). In the Cayo District, forest clearance was concentrated in the northern half of the district for the purposes of agriculture. Most of the area includes the Mennonite settlements north of the Western Highway (White et al. 1996).

In Belize, types of deforestation range from large scale mechanized clearing of forest for agricultural purposes to smaller, slash-and-burn type clearings for temporarily shifting cultivation known as milpa. More than 6,000 persons practice milpa farming countrywide, but it is used predominately by Maya Mopan and Ketchi peoples located primarily in the Cayo and Toledo Districts. This practice involves forest clearing, burning the area towards the end of the dry season and planting corn and/or rice when the rain starts. It is estimated that 7.1 acres of forest are cleared per farmer per year. The land is used for a maximum of two years then left fallow. The standard length of time of the fallow period is 5.2 years but is declining due to increasing demands for food. Although individual milpas are small in area, collectively they can form patchworks that cover large areas (White et al. 1996). Milpa farming has resulted in land clearing on the fringes of forest reserves and on hillsides in the Stann Creek and Cayo Districts. Both milpa farming and citrus cultivation have increased the vulnerability of the soil to erosion and contributed to the degradation of rivers and streams in these areas.

The Forestry Department assessed in 1993 that agricultural expansion was not likely for the main export crops and that future production increase (and forest clearance

required) would tend to occur where the mechanized systems were in place in the northern and central parts of the country. This did not take into account the needs of the small farmers and/or immigrants who do not have ready access to land. Increasing land pressures in areas where the rural population is concentrated are leading to shorter fallow cycles and declining yields. These trends increase the amount of land needed to maintain production. This is most apparent in the communities where the rising trends in rural population growth are greatest, especially in the Cayo District (Central Statistical Office 2000).

The environmental effects of land clearing for agriculture include but are not limited to: higher and greater risks of flooding along rivers due to bank erosion and reduced flow by bulldozed forest debris; contamination of freshwater by agrochemicals; and eutrophication of waterways by discharge from food and sugar processing plants. In addition, such landscape changes may influence the growth of certain vegetation types, potentially leading to changes in vector-borne diseases due to vector habitat formation.

Malaria Epidemiology and Control in Belize

An estimated 1 million cases of malaria are reported each year from the Americas (WHO 1995). The Pan American Health Organization, in 1994, classified Belize as the only country in Latin America to be at “high risk” for malaria transmission. During 1994, Belize reported 9,957 cases of malaria, with the majority coming from the districts of Cayo, Corozal and Toledo. These districts still account for the majority of all reported cases (see below). At the time when systematic collections of malaria data started in 1959, the number of *Plasmodium falciparum* cases was three times as high as the reported *P. vivax* cases (PAHO 1992). The predominate malaria parasite currently is *P.*

vivax. However, low-level transmission of *P. falciparum* still occurs. It is believed that *P. falciparum* cases originate in adjacent countries (MOH). In 1987, seven cases of *P. malariae* were diagnosed (Turner 1988), but none have been reported since.

Malaria case detection in Belize relies heavily on passive case detection, with ill patients reporting to trained village health collaborators or to the district hospitals (MOH). Everyone presenting with symptoms of malaria at local clinics is given presumptive treatment with chloroquine and a thick and thin blood film is made. All slides are sent to a central hospital (Karl Heusner Memorial Hospital) in Belize City for positive confirmation. Upon parasite confirmation, the Vector Control Office within the political district from which the slide was sent is notified, and a Vector Control Officer then visits the patient at his/her village to give the remaining course of treatment. If the locality of a case presents problems of continuing access for the successive treatments, the first dose is administered and the remainder handed over with adequate instructions for self-administration by the patient or a guardian. If *P. falciparum* is diagnosed, the village is targeted for immediate residual spraying. There have been no official reports of chloroquine resistance for either parasite species to date.

Malaria control in Belize has historically focused on the spraying of houses with insecticides. An organized nationwide program using DDT officially started in 1957 as part of the Global Malaria Eradication Campaign (WHO), and by 1961 only 23 malaria cases were reported countrywide (Brown et al. 1976). This program continued until 1989, when outside pressure from international agencies forced the Vector Control Program (VCP) to reduce the use of DDT in house-spraying (Attaran et al. 2000). From 1989-1991, there was intermittent spraying, and from 1993-1995 most house spraying in Belize

had completely stopped due to a ban on DDT use for malaria control. Malaria prevalence began to increase starting in 1991 and continued until 1995. During 1993, there was an incidence rate of 42 cases per 1000 individuals (WHO 1996, 1997). The total number of cases in 1994 rose to 9,957 (47.4 cases per 1000 individuals) and by 1995 malaria prevalence had reached 5% of the population per year, the highest rate since the Pan American Health Organization began collecting malaria statistics in 1959. This represented a 64% increase in malaria cases so that in 1996, a house-spray program was initiated using both DDT and deltamethrin. The re-initiation of a house-spray program resulted in a decrease of more than 40% in reported malaria cases from 1995 to 1996.

Presently, the Ministry of Health uses deltamethrin for their house-spray program. Homes are routinely sprayed bi-annually (i.e., before and after the wet season) within those five villages contributing to the highest malaria prevalence within each political district. Additional ultra-low volume (ULV) treatment with malathion is utilized during semi-routine schedules within the wet season throughout all major cities countrywide. This is performed primarily for dengue control, but is also employed for anopheline control. Anopheline larval control does not comprise a large component of the malaria management program, but under conditions of high populations and/or malaria transmission, the application of oils to potential breeding habitats surrounding homes will be performed. Unfortunately, instability in resources including manpower, equipment purchase/maintenance and necessary transport has greatly stressed control operations (pers. observ.).

Malaria transmission remains low with a total of 1,092 cases reported for the entire country in the year 2001 and 951 in 2002 (MOH). Despite the low number of total

cases, it should be understood that the entire country is at risk due to demographics (see above). *Plasmodium vivax* is the predominant species of parasite, with *P. falciparum* infection occurring infrequently (i.e., only 6 cases in 2001 and 0 reported in 2002). Malaria case distributions for both years had a clear demarcation between the northern (Corozal, Orange Walk and Belize Districts) and central/southern (Cayo, Stann Creek and Toledo Districts) generalized ecoregions (Figure 6), with only 13.1% (143) cases being reported in the north and 86.8% (948) reported in the central/southern districts for 2001. Similar case patterns were seen in 2002, with 17.8% (169) reported in the northern districts and 82.2% (781) in the central/southern districts (MOH). Malaria cases in Belize are reported throughout the year (Figure 7). However, the seasonal distribution of malaria prevalence can vary between districts and reflects the multiple complexities of disease transmission that include variations in the individual vector ecology and environmental patterns found within each district.

Vector Studies in Belize

Combined anopheline surveys throughout Central America and Mexico have identified 14 species (two genera and 5 subgenera) that inhabit the country of Belize (Wilkerson et al. 1990). The public health importance of malaria in Belize has led to previous investigations focusing on the roles and efficiency of four primary anopheline species which have been incriminated in the transmission of malaria within the region including: *An. albimanus* Weidemann, *An. pseudopunctipennis* Theobald, *An. vestitipennis* Dyar & Knab and *An. darlingi* Root. Other than historical observations (Kumm and Ram 1941; Komp 1942), the bionomics of these species in Belize was not documented until the research conducted throughout the past decade (Rejmankova et al.

1993; Roberts et al. 1993; Manguin et al. 1996; Bangs 1999; Grieco 2000; Grieco 2001).

Other anopheline species found at the present research site include: *An. apicimacula* Dyar and Knab; *An. crucians* Wiedemann; *An. gabaldoni* Vargas; and *An. punctimacula* Dyar and Knab. In addition, *Chagasia bathana* Dyar and aberrant morpho-types of *An. darlingi* (Harbach et al. 1993) were also collected. The following are brief descriptions of the bionomics of three primary anopheline species that have to date been the focus of recent malaria transmission research within Belize.

Anopheles albimanus

Throughout its geographic distribution (Figure 8), extending from Mexico to northern South America and the islands of the Caribbean, *An. albimanus* is considered one of the predominant species causing malaria transmission (Komp 1942; Horsfall 1955; Ramsey et al. 1994; Faran 1980; Breeland 1980). *Anopheles albimanus* is primarily characterized as being a lowland species in coastal areas (Rubio-Palis and Zimmerman 1997).

Kumm and Ram (1941) first documented the presence of *Anopheles albimanus* in Belize and Gabaldon (1949) described *An. albimanus* as inhabiting coastal areas in sunlit pools of freshwater. More recent studies have shown this vector to be the most ubiquitous anopheline species in Belize. Primarily associated with cyanobacterial mats and habitats with precipitated levels of calcium carbonate (Rejmankova et al. 1993, 1995), *An. albimanus* has also been found in riverine and tall dense macrophyte marshes (Grieco 2001), demonstrating the ability of this species to breed in a variety of habitats. *Anopheles albimanus* populations in Belize are highest during the wet season of June-December.

Because of its ubiquitousness, *An. albimanus* was once considered to contribute the most to malaria transmission in Belize. Roberts et al. (1996) surveyed 15 villages in central and southern Belize to determine which anopheline species were prevalent and responsible for malaria transmission. In these studies, *An. albimanus* represented 78% of all specimens collected. However, behavioral studies in Belize have shown adults to exhibit a weak endophagic (indoor) feeding behavior with an outdoor:indoor ratio of 1.0:0.21 and lower (Roberts et al. 1993; Bangs 1999; Roberts et al. 2000). In addition, relatively low numbers of females were found biting indoors throughout the night (Bangs 1999), and *An. albimanus* populations in Belize have been shown to feed predominately on non-human hosts (Grieco 2001). These behavior patterns are similar to those reported in other studies throughout Central America (Elliott 1969, 1972; Garrett-Jones 1964; Breeland 1972; Garret-Jones et al. 1980; Frederikson 1993).

Examination of wild-caught *An. albimanus* mosquitoes from Belize for sporozoite infection using the enzyme-linked immunoassay (ELISA), showed a minimum field infection rate (MFIR) lower than both *An. darlingi* and *An. vestitipennis* (Achee 2000). In addition, 6 of the 8 *Plasmodium vivax* and both of the *P. falciparum* positive *An. albimanus* were collected from outside the house. When membrane fed a non-endemic laboratory strain of *P. falciparum*, *An. albimanus* exhibited no salivary gland infection (Grieco 2001). Infection studies of *An. albimanus* in other geographic regions reported the adults of particular larval strains to exhibit a higher infection rate with *P. vivax* than with *P. falciparum* (Gonzalez-Ceron et al. 2000; Warren 1997). It is thought that this vector is effective in malaria transmission only at high population densities (Elliott 1972; Loyola et al. 1993). Such studies are revealing that while *An. albimanus* contributes to

malaria transmission in Belize, this species is not as important as other anopheline species within the country.

Anopheles vestitipennis

Anopheles vestitipennis can be found throughout the coastal region of Mexico, Central America (Wilkerson et al. 1990), regions of northern South America, Cuba and Puerto Rico (Loyola et al. 1991; Mekuria et al. 1991; Padilla et al. 1992; Marquetti et al. 1992) (Figure 9). It is most common along regions of the Atlantic and Gulf coast but can be found in large numbers throughout the Yucatan and Guatemala (Loyola et al. 1991; Arredondo-Jimenez et al. 1996). Within its range, *An. vestitipennis* has been found breeding in clean, heavily shaded habitats with emergent vegetation (Komp 1942; Horsfall 1955; Belkin et al. 1965; Loyola et al. 1991). Recent studies in Belize have described associations between *An. vestitipennis* and both flooded forests and tall-dense macrophyte marshes with populations most abundant during the wet season (Rejmankova et al. 1998; Roberts et al. 1993; Grieco 2001).

This vector has only recently been considered an important species involved with malaria transmission in Belize. Behavioral studies conducted by Roberts et al. (1993) and Grieco (2000) have described *Anopheles vestitipennis* biting in higher densities within homes than outdoors throughout the night. Similar endophilic and endophagic behaviors have been described in Mexico (Loyola et al. 1991), Costa Rica (Kumm et al. 1940) and Guatemala (Richards et al. 1994). In addition, examination of host-feeding preferences indicates that *An. vestitipennis* preferred to feed on humans rather than other animals (Grieco 2001). Human-preference feeding behavior was also reported by Mekuria et al. (1991) in the Dominican Republic, and *An. vestitipennis* has also been shown to partake

in multiple blood feeding habits (Arredondo-Jemenez et al. 1998); both behaviors defining an extremely competent vector.

Positive immunoassays for *P. vivax* in wild-caught *An. vestitipennis* populations from Chiapas, Mexico have been described (Loyola et al. 1991). Historical studies by Kumm and Ram (1941) found one natural *Plasmodium* sporozoite infection in a female specimen from Belize. In a recent study of wild-caught females collected in Belize from 1994-1997, a higher minimum-field infection rate (MFIR) was found for *An. vestitipennis* (0.282%) than either *An. albimanus* (0.126%) or *An. darlingi* (0.271%) (Achee et al. 2000). In addition, both positive samples were infected with *P. falciparum* and were collected from inside homes.

Anopheles darlingi

Anopheles darlingi was first described by Root in 1926 from Rio de Janeiro in Brazil. Davis (1931) first incriminated *An. darlingi* as a vector of malaria in Para State of Brazil based upon finding sporozoites in the salivary glands of dissected specimens.

Anopheles darlingi is found from Southern Mexico to northeastern Argentina with a lack of official reporting from Nicaragua, Costa Rica or Panama (Manguin et al. 1996; Linthicum 1988) (Figure 10). *Anopheles darlingi* is considered the most efficient malaria vector in the New World (Foote and Cook 1959) and, where it occurs, has been found to be the major or only vector of human malaria in South America (Forattini 1962; Lourenco-de-Oliveira et al. 1989). Komp (1940) was the first to report the presence of *An. darlingi* in Belize, and Kumm and Ram (1941) later confirmed these findings. However for 50 years, adult and larval sampling was unsuccessful in detecting this vector in Belize until recently (Harbach 1993; Roberts et al. 1993).

Anopheles darlingi is associated with riverine habitats throughout its geographic distribution (Faran and Linthicum 1981). Studies in South America have found *An. darlingi* larvae in shaded areas of debris and floating vegetation in rivers where surface flow is impeded (Fleming 1963; Panday 1980; Hudson 1984; Tadei et al. 1998). Strong tidal action of rivers close to the coast has been shown to be unfavorable for larval habitats in Suriname (Rozendaal 1990). Limited research has been conducted on the larval ecology of *An. darlingi* in Belize (Harbach et al. 1993; Manguin et al. 1996; Rejmankova et al. 2000) since its first discovery in the 1940's. Manguin et al. (1996) studied the ecological determinants of larval habitats to include floating mats of debris in shaded areas along freshwater river margins. In particular, the floating mats were associated with overhanging bamboo and submersed roots along the riverbank.

A review of the adult biology of *An. darlingi* has been previously published (Charlwood 1996). *Anopheles darlingi* has been described as both exhibiting a strong attraction for human hosts and having an endophagic feeding behavior in several endemic regions (Deane et al. 1946; Roberts et al. 1987; Klein and Lima 1990; Rozendaal 1989). Studies using human-baited biting collections in Belize have shown that *An. darlingi* feeds in equal numbers indoors and outdoors of houses but exhibits a higher endophagic behavior than either *An. vestitipennis* and *An. albimanus* (Grieco 2001; Roberts et al. 1996). Data from these studies, however, are only representative of early evening activity (6:30-8:00 p.m.) with no late-night biting cycles determined. This reported endophagic response might be different if based on whole night collections.

Until the present study, there have been no studies conducted in the Cayo District of Belize to define changes in *An. darlingi* population densities throughout the seasons.

In Suriname, Rozendaal (1990) showed *An. darlingi* larval habitats to be highly associated with rainfall and river levels. During the rainy season, *An. darlingi* was found in vegetation and floating detritus in flooded forest areas and other flooded depressions near rivers where the riverbanks have overflowed (Rozendaal 1987, 1992). Adult populations and malaria transmission were highest during the rainy season. Dry season breeding sites consisted of creeks and between roots and fallen trunks along river margins (Rozendaal 1990). Rozendaal (1992) described how periods of heavy rain, in the interior regions of the Amazon rainforest, would reduce *An. darlingi* breeding habitats in the river by the flushing actions of rising river levels. Adult populations would increase only after the river receded during the transitional months between the wet and dry seasons, corresponding with peaks of regional malaria transmission (Ferraroni and Hayes 1979; Charlwood 1980).

Kumm and Ram (1941) were the first to document the presence of sporozoites in salivary gland dissections of *An. darlingi* collected from Belize. In a survey of anophelines conducted in Belize from 1994 to 1997, Achee et al. (2000) demonstrated that *An. darlingi* had a higher MFIR (0.271%) than *An. albimanus* (0.126%). This same study showed a *P. falciparum* positive pool occurred in a sample of *An. darlingi* that was obtained from an indoor biting collection. Other studies from South America have demonstrated the high susceptibility of this vector to *P. falciparum* compared to other anopheline species (Klein and Lima 1990).

Although an important aspect in malaria epidemiology, relatively few studies have examined the flight behavior of *An. darlingi*. Mark-release-recapture studies on *An. darlingi* in Rondonia, Brazil showed a maximum flight distance of 7.2 km from the

release site (Charlwood and Alecrim 1989). Another study in Brazil reported a flight range of up to 2 km (Deane et al. 1948). The flight behavior of *An. darlingi* in Belize is presently unknown. The present study is the first to describe the recapture rate of this vector at various distances from a fixed release site.

Remote Sensing and GIS Applications in Belize

The ability to conduct disease risk assessments using satellite imagery (remote sensing) is due to the basic relationship that all arthropod-borne diseases are integrally related to the surrounding landscape ecology (Pavlovsky 1960). Each vector has a specific habitat defined by water, vegetation, and other factors. These environments can be detected by satellite sensors that record spectral values at various electromagnetic wavelengths (i.e., light, heat or microwave). Spectral characteristics are recorded as numeric values and can be viewed as individual black and white images or as a color composite in an image processing software program.

There are many different types of satellite sensors that can be used in remote sensing studies (e.g., multispectral, panchromatic, radar, etc.). Each has its own advantages and limitations (Beck et al. 2000). The type of sensor a study uses will depend on the scale of area to be investigated, land cover type, and budget. For example, the LANDSAT multispectral sensing system with a spatial resolution of 15-30 meters/pixel can be used for regional studies, while the SPOT and IKONOS sensing systems with 10-20 meters/pixel and 1-4 meters/pixel, respectively, are better suited for local studies. Furthermore, if a study area consists of dense vegetation or cloud cover, the RADARSAT sensing system may be necessary because it can penetrate through these conditions.

While remote sensing provides the tools for visualizing a study area, geographical information systems (GIS) manage, analyze and display spatial data. The spatial data, defined by geographic coordinates, can become an overlay onto remotely sensed images or digitized topographical maps. The researcher can then determine high-risk areas for disease transmission based upon specific parameters such as proximity to certain land cover types and known vector bionomics (Clarke et al. 1996).

Both remote sensing and GIS applications have been used to research malaria in Mexico (Roberts and Rodriguez 1994; Andre 1994). Similar studies have been conducted in Belize to predict the presence of adult vectors of importance. SPOT (20-m resolution) satellite imagery was able to predict *An. pseudopunctipennis* based on defined ecological determinants of larval habitats with 50% and 100% accuracy for high and low probability sites, respectively (Roberts et al. 1996). Studies have also examined the use of SPOT satellite imagery to detect *An. vestitipennis* and *An. punctimacula* larval populations (Rejmankova et al. 1998). In addition, *Anopheles albimanus* adult densities were predicted with 100% and 89% accuracy within villages at varying distances from remotely sensed larval habitats using Landsat (30-m resolution) imagery (Rejmankova et al. 1995). Until the present research, no targeted analysis has been conducted to determine the predictive capability of remotely sensed land cover and river characteristics to determine high-risk areas for *An. darlingi* larval habitats.

Study Site

Preliminary mosquito collections were performed throughout the country to determine locations that exhibited *An. darlingi* populations at density levels adequate for study (Appendix 1). A study site was established at 17°09'59.5"N and 88°36'09.6"W

within the central Cayo District of Belize on the property of Mr. Ramon Galvez Sr. The site was approximately 12 miles south of the capital of Belize, Belmopan, and an estimated 6 miles from the intersection of the Hummingbird Highway and the Hummingbird Citrus Limited (HCL), formally Hershey's Plantation (Figure 11). An observational census of terrestrial animals includes: coral and fer-de-lance snakes; iguana; koatomundi; deer; tapir; porcupine; opossum; gbnut; yellow-headed parrots; toucans; howler monkeys and jaguar. Total rainfall reported at Hershey's Plantation for 2001 was 2,461 mm and 3,236 mm for 2002 (National Meteorological Service). The majority of rains, 1,937 mm and 2,696.5 mm, fell during the typical rainy season (June-December) of both 2001 and 2002, respectively.

The study site is in association with the mid-reaches of the Sibun River Watershed, in which there are a total of eleven villages, with the three closest to this location being Armenia (pop. 400), Caves Branch (pop. 70) and Hershey's Plantation (pop. 100). The majority of inhabitants are Spanish immigrants with some indigenous Maya and Creole. Census data from 2000 described the Cayo District with the highest number and proportion of foreign-born persons compared to any of the other districts. For every 5 persons in Cayo, at least one of them is foreign-born, with Guatemala accounting for 51.0%, El Salvador 24.1% and Honduras 4.9%. This district attracts immigrants mainly for the economic activities in the citrus and banana industries as well as access to land for subsistence farming.

The surrounding landscape includes the foothills of the Maya Mountain Range, secondary broad-leaf forest and agriculture (including milpa, citrus and pasture). The Sibun River originates in the Maya Mountains to the West and traverses over 100 km

through pine forests, savannah, low-lying lagoons and mangrove swamps and finally empties into the Caribbean Sea to the East. The headwaters are protected by the Sibun Forest Reserve status, which are characterized by low populations and few milpa farms. The river then flows through the limestone plain of central Belize characterized by thicket, herbaceous scrub and broadleaf forest. Several citrus plantations are also located in this region. The input of several tributaries has made this area prone to frequent flooding, which creates an alluvial plain ideal for agriculture. Where the Cave's Branch Tributary joins the Sibun River and nears the Western Highway, more frequent settlements can be found in the savannah and thicket habitat north of the river. Near the mouth, approximately 20 km south of Belize City, the riverbank drops and the vegetation changes from fig and spiny bamboo to mangrove swamps.

The hydrology of the Sibun River and its tributaries has not been investigated in depth due to the dynamic characteristic of the river. River width and depth vary tremendously both within and between seasons as does the flow rate, especially along the mid-reaches of the river. The most extensive information available on the Sibun is the daily river level data collected by the Hydrology Department of the Belize Government at the Freetown Village gauge. These data have been collected twice daily on an annual basis since June 1981. Unfortunately, data only represents the lower reaches of the river and while useful for overall river level fluctuations, does not provide a detailed description of changes occurring at other regions of the river. Data from Freetown show the high flow season ranges from June-December (corresponding to wet season precipitation patterns) but sometimes begins in May and extends into February.

Citrus plantations, milpa farming, pastureland and gravel mining have a negative impact in the mid-reaches of the Sibun River. Several locations along the riverbank demonstrate the infraction of the 66-foot buffer zone for forest clearance detailed in the Forest Preservation Act. In addition, gravel mining of the riverbed during the dry season has reduced water quality by increasing sedimentation and modifying the flow of water. Because most of the inhabitants in the area are immigrants, milpa farming practices have also led to soil erosion along the banks.

Malaria case distribution for those villages surrounding the study site showed 7 total cases of *P. vivax* for the year 2001 and 8 total cases of *P. vivax* in 2002 (MOH). No cases of *P. falciparum* cases were recorded for either year. The majority (4/7) of cases in 2001 were detected during the month of July with one case each occurring in May, August and November. For 2002, half of all the reported cases occurred in March with two cases occurring in February and one each in June and September. As is typical throughout the country, malaria cases are passively detected through the Vector Control Office at the hospital located in Belmopan. Reported cases are probably an underestimate of the true prevalence within the immediate locale.

Purpose and Objectives of the Present Study

The overall objective of the present research is to provide to public health officials in Belize the ability to understand the role of *An. darlingi* in endemic malaria transmission and to determine the applicability of utilizing remote sensing and GIS technologies to predict high-risk areas for *An. darlingi* breeding habitats and, therefore, adult population densities. The information gathered during this research should prove applicable in decision-making processes regarding malaria control efforts in Belize.

Specific goals were to: 1) characterize the biting pattern and seasonal distribution of adult *An. darlingi* populations; 2) define the flight behavior of *An. darlingi* at various distances from a fixed release point; 3) determine the role of bamboo in *An. darlingi* larval habitat selection; 4) determine if remotely sensed land cover along riverbanks can be used to predict areas of bamboo growth; and 5) characterize the landscape features associated with *An. darlingi* habitats and determine if remotely sensed river characteristics, house locations and/or land cover can be used to predict larval habitat presence.

These goals were outlined in response to the general hypotheses that: 1) the adult bionomics of *An. darlingi* allows for high vectorial capacity due to this species' anthropophilic, endophagic and late-night biting behavior patterns; 2) deforestation along river margins increases the formation of *An. darlingi* breeding habitats by promoting the growth of overhanging bamboo; and 3) remotely sensed environmental variables can be used to predict high-risk areas within rivers for *An. darlingi* larval habitats.

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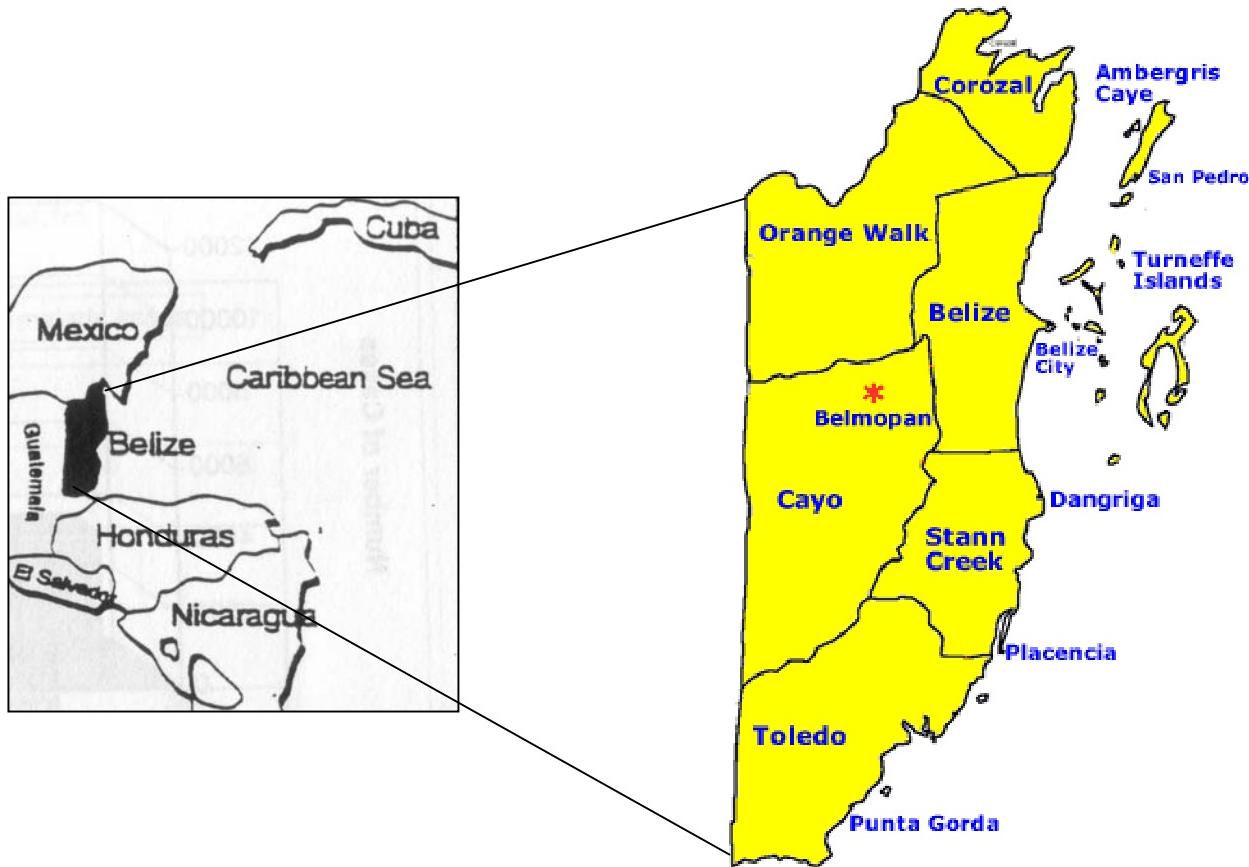


Figure 1. Map of the Central American country of Belize showing the six political districts.

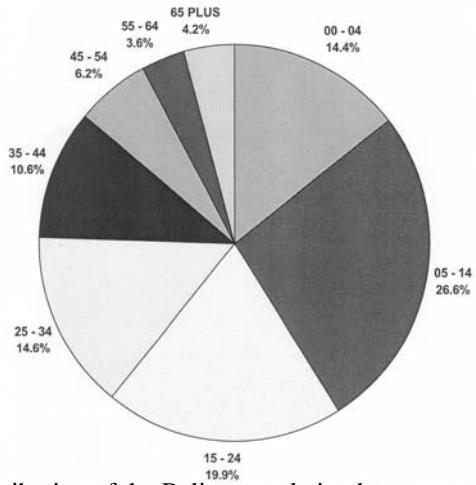


Figure 2. Percent distribution of the Belize population by age group (Central Statistical Office 2000).

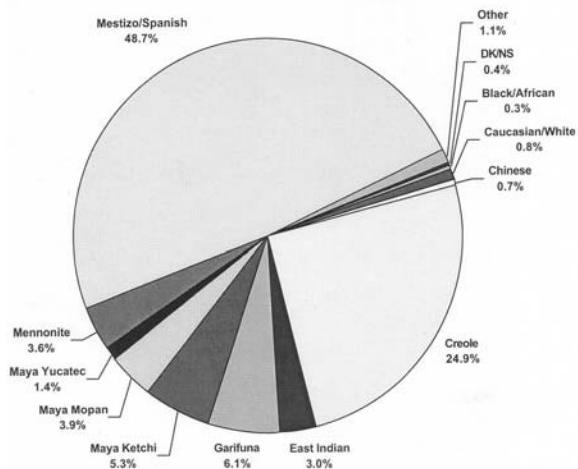


Figure 3. Percent distribution of the Belize population by ethnicity (Central Statistical Office 2000).

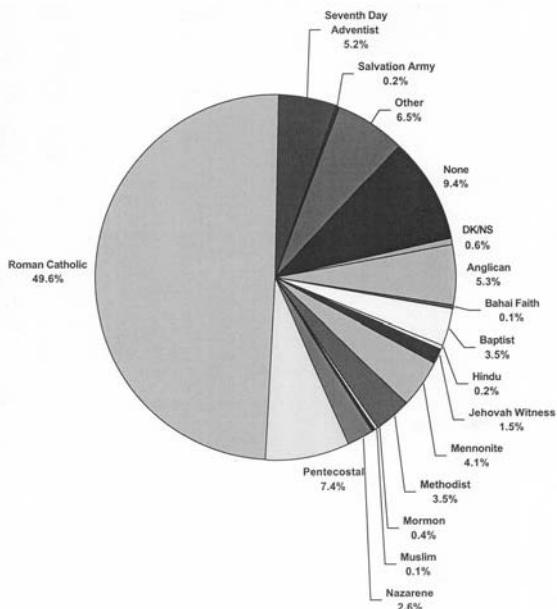


Figure 4. Percent distribution of the Belize population by religion (Central Statistical Office 2000).



Figure 5. Location of forest and associated woodland cover losses on mainland Belize from 1989/92 to 1994 (Land Information Center).

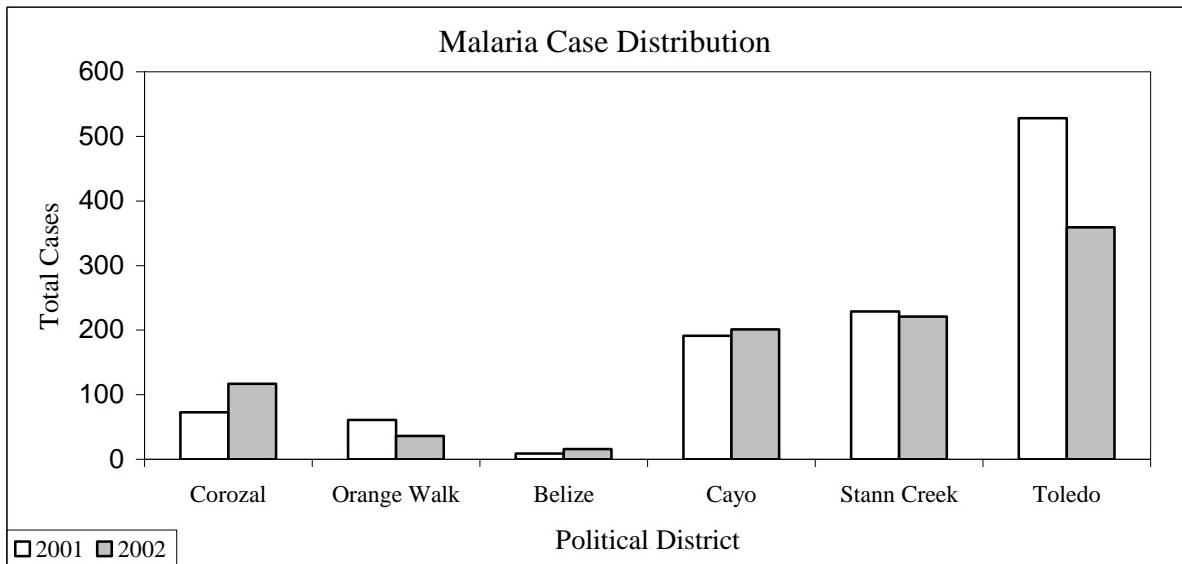


Figure 6. Malaria case distribution by political district for the years 2001 and 2002 (Ministry of Health).

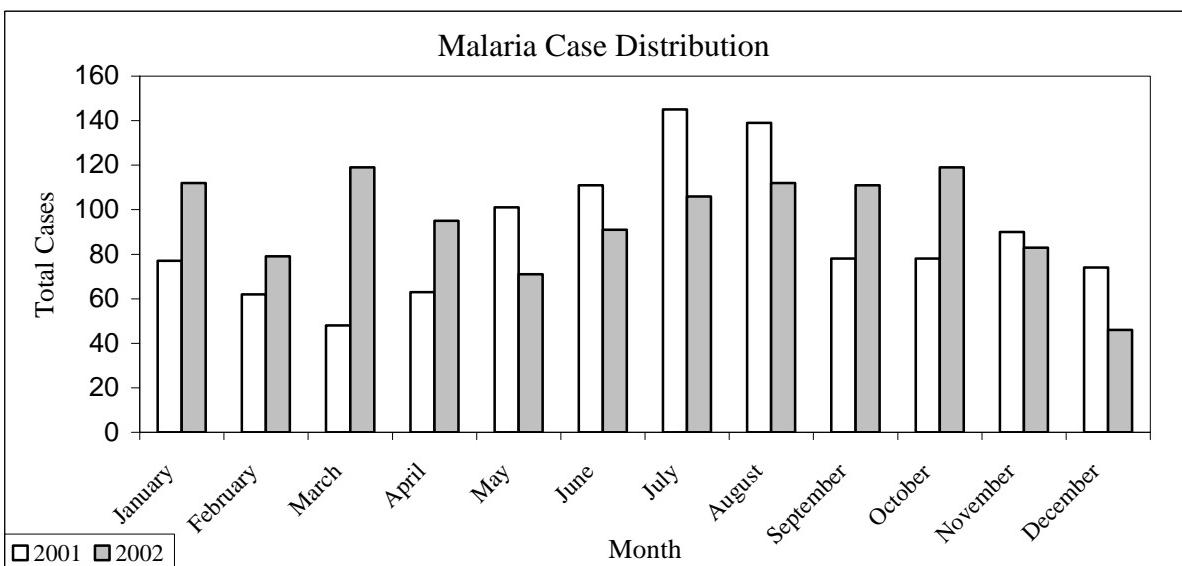


Figure 7. Countrywide monthly malaria case distributions for the year 2001 and 2002 (Ministry of Health).

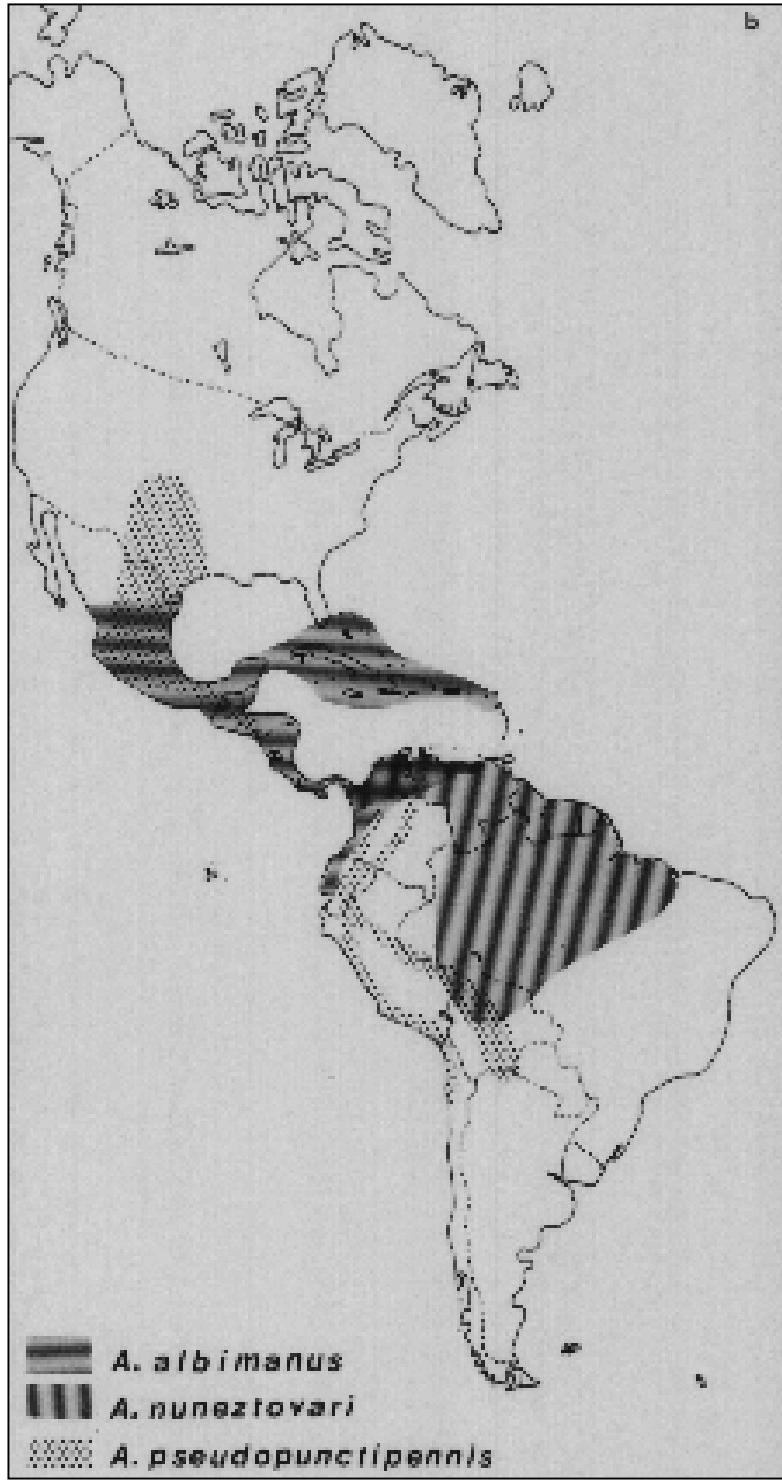


Figure 8. Map of the geographic distribution of *Anopheles albimanus* in the Americas (WHO 1989).

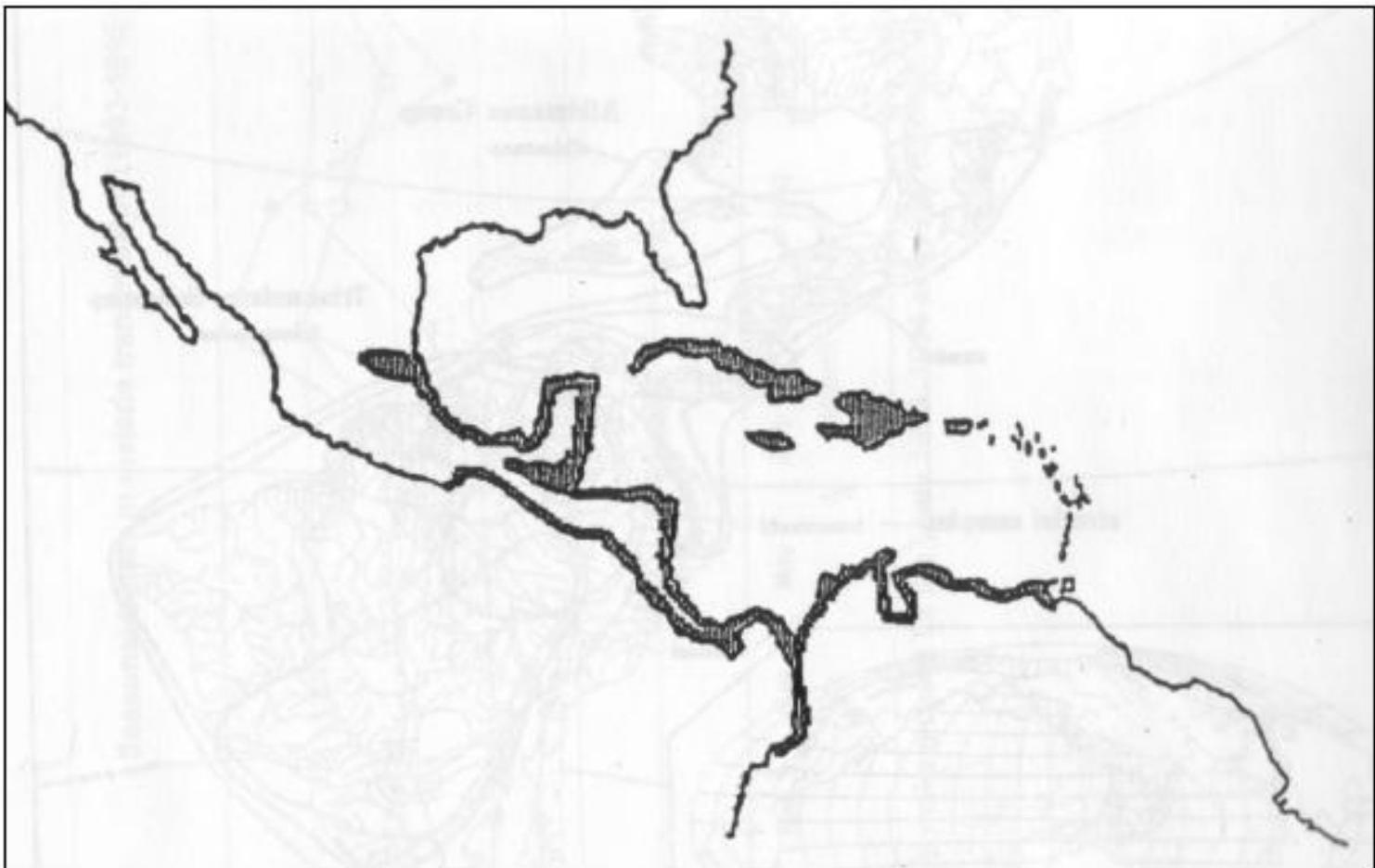


Figure 9. Map of the geographic distribution of *Anopheles vestitipennis* in the Americas (Arrendondo-Jimenez et al. 1980).

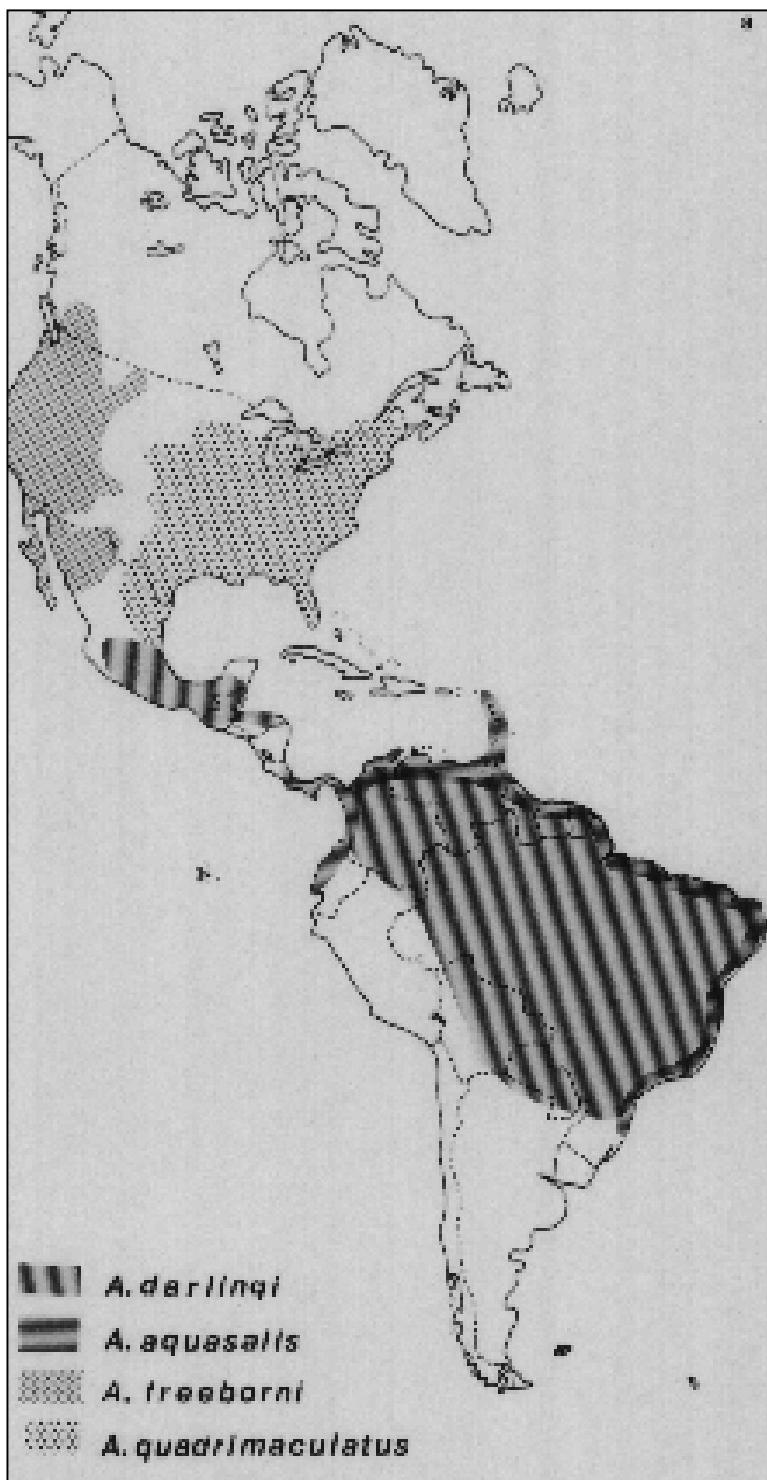


Figure 10. Map of the geographic distribution of *Anopheles darlingi* in the Americas (WHO 1989).

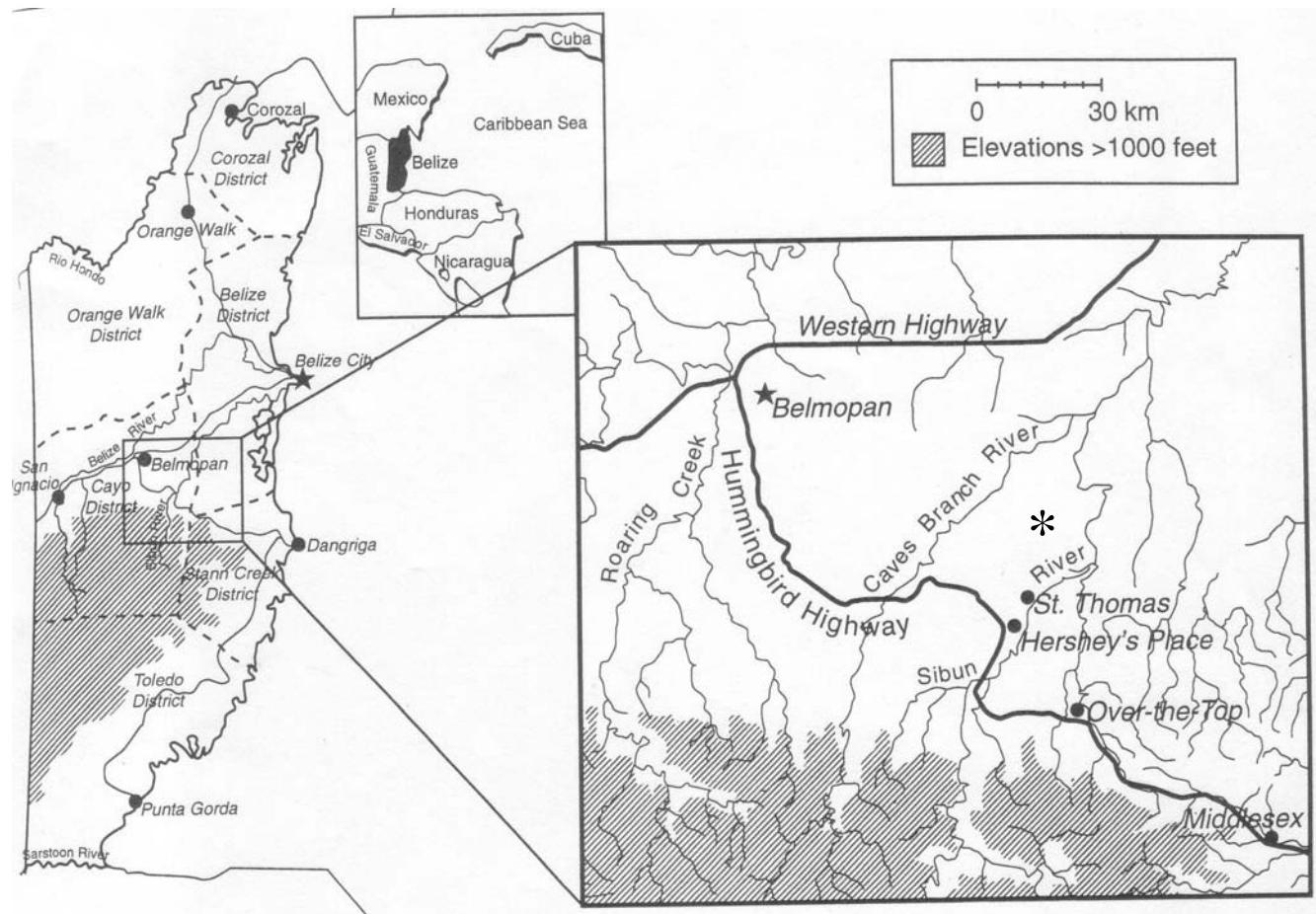


Figure 11. Map of the research site (*) within the centrally located Cayo District (Roberts et al. 1996).

Chapter 2

**The nightly biting patterns and seasonal population densities of *Anopheles darlingi*
in the Cayo District of Belize, Central America**

ABSTRACT

Determining the role of specific local anopheline species in malaria transmission is vital to the success of any malaria control program. This includes defining nightly biting patterns, the ratio of indoor and outdoor biting populations and changes in vector population densities between seasons. The present study utilized a portable experimental hut to conduct human-baited landing collections in order to characterize the biting patterns and seasonal population density changes of the malaria vector *Anopheles darlingi* in the centrally located Cayo District of Belize, Central America.

A total of 25 all-night collections (i.e., sunset to sunrise) were conducted from January 2002-May 2003, capturing a total of 18,878 *An. darlingi* females. *Anopheles darlingi* exhibited a bimodal nightly biting pattern with a predominate peak occurring three hours after sunset and a smaller peak at one hour prior to sunrise. Biting females were collected throughout the night in higher densities indoors (9,611) than outside (9,267) the experimental hut (I:O=1.00:0.96).

Seasonal adult collections show *An. darlingi* population densities were highest during the transitional month between the end of the wet and beginning of the dry season (January) and the end of the dry season and beginning of the wet season (May). A total of 2,010 *An. darlingi* females were captured in 31 two-hour, human-baited landing collections performed from January-October 2002. Monthly total averages were: January=110, February=20, March=23, April=44, May=310, July=99, August=24, September=27, October=44. No collections were performed in the months of June, November and December. *Anopheles darlingi* monthly population densities were found to have no significant associations with high or low temperatures, precipitation or river

level. However, qualitative data examination indicates a negative relationship between river level and *An. darlingi* adult collections suggesting an adverse disturbance of larval habitats. None of the adult *An. darlingi* specimens tested positive for *Plasmodium* spp. infection using the VecTest™ rapid diagnostic kit, and no associations were found between monthly malaria case frequencies and *An. darlingi* adult collections.

INTRODUCTION

Vector behavior influences the dynamics of malaria transmission by governing the intensity, time and place of host-vector interactions (Elliott 1972). Determining peak biting times and location relative to houses is a vital component in implementing successful vector and malaria control techniques. In addition, knowledge of these behaviors in combination with temporal vector distributions and natural sporozoite infection rates provides a better understanding of the vector competence of individual species for a given endemic area. Such studies have traditionally utilized human-baited catches at experimental huts (Service 1993).

The public health importance of malaria in Belize has led to previous investigations characterizing the vectorial roles and efficiency of some of the *Anopheles* species found throughout the country including: *An. albimanus* Weidemann, *An. darlingi* Root, *An. pseudopunctipennis* Theobald, and *An. vestitipennis* Dyar & Knab. While all of these species have been shown to be competent vectors in the transmission of malaria in the Americas (Loyola et al. 1991; Padilla et al. 1992; Ramsey et al. 1994; Lourenco-de-Oliveira 1989; Klein et al. 1991) and specifically Belize (Achee et al. 2000; Grieco et al. 2000), *An. darlingi* is presently considered one of the most important. This consideration is based on this species' characteristics of being anthropophilic, exhibiting

an endophagic feeding behavior, and natural malaria infectivity rates (Kumm and Ram 1941; Roberts et al. 1987; Achee et al. 2000; Grieco 2000).

Despite the extreme importance of *An. darlingi* in malaria transmission, this vector's bionomics has been the least studied in Belize (Manguin et al. 1996; Harbach et al. 1993; Roberts et al. 1996; Roberts et al. 2002; Rejmankova et al. 2000; Achee et al. 2000; Grieco 2000). Previous studies using human-baited biting collections in Belize have shown that *An. darlingi* feeds both outdoors and indoors of houses but exhibits a higher endophagic behavior (Grieco 2000; Roberts et al. 1996; Roberts et al. 2002). Data from these studies, however, were representative of only early evening activity (6:30-8:00 p.m.) with no late-night biting cycles being determined. Host seeking by vectors includes activation of circadian host-seeking behavior. Once activated, host seeking includes vector movement, host detection, house entering, resting and biting. The temporal pattern of these responses may be altered due to host defensive behaviors and environmental parameters that change over the course of the night. Such modifications will alter the biting pattern of vectors. Until the present study, the host seeking and feeding behaviors of *An. darlingi* in Belize throughout the night were unknown.

Because the cycles of arthropod populations are affected by environmental conditions, changes in such parameters will be reflected in variations of vector densities. Within tropical regions, these variations in *An. darlingi* populations often correlate with wet and dry season malaria case distributions (Rozendaal 1992; Tadei et al. 1998; Lourenco-de-Oliveira et al. 1989). Roberts et al. (2002) defined the spatial distribution of adult *An. darlingi* in both riparian and upland zones during both dry and wet season within a two-year period. Results showed *An. darlingi* females to be present in riparian

associated houses during both seasons but were only present at upland homes during the wet season. A seasonal distribution study of *An. darlingi* in Belize indicated adult population densities in the southern Toledo District to be highest during the months of the dry season with lowest rainfall (Grieco 2000). Because specific vectors require specific types of aquatic habitats, temporal distributions will vary within different topographical environments as a result of habitat availability. For this reason, further research examining the relationship between seasonal changes and *An. darlingi* abundance in different regions of Belize was needed.

The following study incorporated the use of an experimental hut to describe the biting behavior of *An. darlingi* in the central Cayo District of Belize. An experimental hut was needed because there is less control over the use of normal, local huts, which can lead to data confounding. For example, the manipulation of windows and doors (i.e., open or close position), the increase in persons indoors (i.e., indoor:outdoor ratio assessment) and, most importantly, local village huts may have been treated with insecticide. However, the results from the present study can be generalized to indigenous structures that have no spray history because care was taken to match the design and construction materials of houses in villages adjacent to the study site (see Chapter 3).

The objectives of the present study were to determine if the reported endophagic response of *An. darlingi* might be different based on all-night collections and to define associations between *An. darlingi* seasonal population densities, environmental data and malaria case frequencies on regional and local scales. It is hoped that this information will provide further insight into the role of *An. darlingi* in the transmission of malaria within the region and aid in disease prevention measures.

MATERIALS AND METHODS

Study Site: The study site was established on open pastureland at 17°09'59.5" N 88°36'09.6" W in the foothills of the Maya Mountain Range along the Sibun River in the central Cayo District of Belize. Located on the property of Mr. Ramon Galvez Sr., the site was approximately 12 miles south from the capital of Belize, Belmopan, and an estimated 6 miles from the intersection of the Hummingbird Highway and the Hummingbird Citrus Limited (HCL), formally Hershey's Plantation (Figure 1). Temperatures range from 29°C in January to 34°C in May (National Meterological Service). An observational census of terrestrial animals includes: coral and fer-de-lance snakes; iguana; koatomundi; deer; tapir; porcupine; opossum; gribnut; yellow-headed parrots; toucans; howler monkeys and jaguar.

The study site is within the mid-reaches of the Sibun River Watershed, in which there are a total of eleven villages, with the three closest to this location being Armenia (pop. 400), Caves Branch (pop. 70) and Hershey (pop. 100). The majority of inhabitants are Spanish immigrants with some indigenous Maya and Creole. Census data from 2000 described the Cayo District with the highest number and proportion of foreign-born persons compared to any of the other districts (Central Statistical Office). For every 5 persons in Cayo, at least one of them is foreign-born with Guatemala accounting for 51.0%, El Salvador 24.1% and Honduras 4.9%. This district attracts immigrants mainly for the economic activities in the citrus and banana industries as well as access to land for subsistence farming.

Experimental Hut: All collections were conducted using an experimental hut designed according to local house construction (Central Statistical Office) and built using locally

acquired materials (see Chapter 3). The experimental hut was necessary to control for previous pesticide treatment as well as number of humans within the collection site. A house survey within the adjacent village of Armenia was conducted prior to the start of the study to ensure comparability of living area and design between the experimental hut and indigenous homes (Appendix 2). The 13 x 13 ft. hut consisted of untreated plank walls, a corrugated zinc roof and dirt floor. There was one window per wall for a total of three windows, each 2 x 2 ft., and a door measuring 6 ft. x 3.5 ft. The windows and door remained open throughout the entire biting collection period.

All-night Mosquito Collections: All-night biting patterns of *An. darlingi* were characterized using 12-hr human-baited collections starting 30 min. prior to sunset and continuing until 30 min. after sunrise. One collector was located outside the hut approximately 7 ft. at a diagonal from the door while another collector was located at the center of the inside of the hut. All anopheline females landing on the exposed lower legs of the collectors were captured using manual aspirators and flashlights during a twenty-minute sampling period each half-hour. Collectors rotated positions (i.e., inside/outside) after each sampling period and were relieved with another set of two collectors after three hours.

Mosquitoes were placed into modified cardboard ice cream pint cartons and killed each hour by suffocation with acetone vapors within an airtight killing chamber (i.e., 1-gal. insulated cooler). Upon completion of the total collection period, specimens were sorted by species (Wilkerson and Strickman 1990), counted and placed into 1.5 ml Eppendorf vials properly labeled with date, indoor/outdoor station and species and then placed over silica gel in a sealed container. The number of each anopheline species

captured hourly at both the indoor and outdoor locations were recorded onto corresponding forms (Appendix 3). Correlations between environmental variables, including indoor and outdoor temperature and relativity humidity (gathered using HOBO® Pro Series Weatherproof Data Loggers (Forestry Suppliers Inc., Jackson, MS)) with the density of *An. darlingi* females was analyzed using nonparametric bivariate statistics and multiple linear regression (SPSS version 9.0, SPSS Inc.).

Seasonal Mosquito Collections: Human-baited landing collections were performed at the same research site as previously described except that each collection lasted only two hours from sunset with collectors rotating indoor and outdoor locations after a 30-min. sampling period. The total number of *An. darlingi* females was recorded onto a corresponding form (Appendix 3) and monthly averages calculated. Nonparametric bivariate correlations and multiple linear regression analyses (SPSS version 9.0, SPSS Inc) were performed to define associations between anopheline populations and environmental data including temperature, precipitation and river level (National Meteorological Service). In addition, qualitative associations between regional and local malaria case distributions (Ministry of Health) and *An. darlingi* population densities were examined.

Natural Sporozoite Infections: Samples of each anopheline species sorted by hour, biting location (i.e., indoor or outside) and date from both all-night and two-hour seasonal collections were screened for natural malaria sporozoite infections using VecTest™ rapid diagnostic kits (Medical Analysis Systems, Inc., Camarillo CA.) according to manufacturer's instructions.

RESULTS

All-night Biting Collections:

From 25 all-night collections performed from January 2002-May 2003, a total of 18, 878 *An. darlingi* female mosquitoes were captured (Table 1). Other anopheline species collected in decreasing frequency include: 527 *An. albimanus*, 371 *An. pseudopunctipennis*, 46 *An. punctimacula* Dyar and Knab, 44 *An. vestitipennis*, 21 *An. apicimacula* Dyar and Knab, 5 *An. gabaldoni* Vargas, 2 *An. punctipennis* Say and 1 *An. crucians* Wiedemann. In addition, there were a total of 2 *Chagasia bathana* Dyar and 258 aberrant morpho-types of *An. darlingi* (Harbach et al. 1993). These aberrant morpho-types were not included in the overall *An. darlingi* population biting pattern analyses.

Anopheles darlingi females were captured biting throughout the night exhibiting a bimodal indoor/outdoor biting pattern with one predominate peak occurring three hours after sunset and a weaker peak defined one hour prior to sunrise (Figure 2A). The cumulative majority of all females captured both indoors (54%) and outdoors (56%) was collected within 5 hrs post-sunset although biting continued throughout the night (Figure 2B). *Anopheles darlingi* exhibited an endophagic biting behavior with a higher population density existing inside the experimental hut (9,611) as compared to outdoors (9,267) giving an indoor to outdoor ratio of 1.00:0.96 (Table 1).

Examination of other anophelines at the research site, which have been shown to be of importance in the country, defined an endophagic behavioral response of *An. albimanus* females (Table 1). This species demonstrated an indoor to outdoor biting ratio of 1.00:0.99. Although total numbers of *An. albimanus* collected were low (527), the all-night collections indicate a bimodal biting activity pattern with an early peak occurring

within two hours post-sunset and another pronounced peak occurring three hours prior to sunrise (Figure 3A). However, the majority of *An. albimanus* females collected, both indoors (55%) and outdoors (58%), were captured within three hours after sunset with minimal numbers biting during the rest of the sampling period (Figure 3B).

Interestingly, a strong endophagic behavior was described for *An. pseudopunctipennis* with 242 females being collected inside compared to 129 outside the experimental hut. This defined an indoor to outdoor biting ratio of 1.00:0.53 (Table 1). While not well defined due to low numbers of collected specimens (371), females exhibited a peak in indoor biting starting two hours post-sunset and continuing until six hours post-sunset with low numbers continuing to bite throughout the night (Figure 4A). Outdoor biting peaked at five hours post-sunset and again during the hour prior to sunrise. The majority of *An. pseudopunctipennis* both inside (54%) and outside (47%) the experimental hut were not collected until five hours post-sunset (Figure 4B).

The total number of other female anopheline species collected at the research site was too low to use in extrapolating meaningful biting activity patterns. However, indoor to outdoor biting ratios were calculated (Table 1). Because of this vector's importance in other studies within Belize, it should be noted that populations of biting *An. vestitipennis* were denser inside (24) than outdoors (20) demonstrating an indoor to outdoor ratio of 1.00:0.83.

Because this is the first report to describe the all-night biting behavior of *An. darlingi* in Belize, further analyses were conducted to determine the differences in biting patterns of the target vector according to lunar cycles. Each of the 25 all-night collections was grouped into a moon phase category based on the lunar cycle of that particular

collection date (National Meteorological Service). A total of 2,491 *An. darlingi* were captured during three collections performed during a new moon, 4,655 during four first quarter moon phase collections, 4,575 in nine collections conducted during a full moon and 2,023 in one last quarter moon phase collection. Similar bimodal peak biting patterns existed for all moon phases (Figure 5A-D). Higher inside than outdoors *An. darlingi* biting populations were exhibited during both full and new moon collections but first quarter (0.95:1.00) and last quarter (0.98:1.00) collections described a slight exophagic behavior.

During full moon phase collections the majority of females were captured one hour earlier (i.e., 4 hours post-sunset) than other lunar cycles (Figure 6A-D). Nonparametric analyses between moon phase categories indicated a significant difference between the numbers of *An. darlingi* collected during a full moon phase compared to other lunar cycles indoors (new: $z=-2.276$, $p=0.023$; first quarter: $z=-2.607$, $p=0.009$; last quarter: $z=-2.031$, $p=0.042$). The same analyses for outdoor biting show similar results except there was no difference between full moon and last quarter collections (new: $z=-3.601$, $p<0.001$; first quarter: $z=-2.521$, $p=0.012$; last quarter: $z=-1.515$, $p=0.130$). No differences were seen between other moon phases. Examinations of *An. darlingi* density levels by collection hour between lunar cycles indicate significantly more captured females during hour 13 for new moon nights for both indoor ($p=0.019$; $p=0.003$; $p=0.037$) and outdoor ($p=0.019$; $p=0.011$; $p=0.037$) collections compared to first quarter, full moon and last quarter lunar cycles, respectively. In addition, the new moon phase had higher outdoor densities during hour 12 than both first quarter ($p=0.001$) and full moon

collections ($p=0.032$). Hour 12 also showed higher outdoor biting between last quarter and full moon collections ($p=0.028$).

Temperature readings during the collections inside the hut averaged 24°C with a range from 17°C to 31°C , while outside the hut temperatures averaged 23°C and ranged from 16°C to 29°C . The average temperature indoors was consistently higher each hour throughout the night compared to the average outside temperature (Students t-test; $p=<0.05$) (Figure 7A). Relative humidity levels recorded indoors ranged from 69%-100% with the average of 90% being significantly lower than the average humidity level of 95% recorded outside the experimental hut (students t-test; $p=0.002$) (Figure 7B). High wind speeds recorded at the collection site averaged 4 km/hr and ranged from 0 to 32 km/hr, with the strongest winds occurring during the first 4 hours post-sunset (Figure 7C). Linear regression analyses of average nightly environmental data with nightly *An. darlingi* densities indicate that wind speed is the only parameter to have a significant effect (negative) on the number of females collected indoors ($t=-2.141$; $p=0.050$). Outdoor biting was not significantly associated with any of the environmental variables.

Seasonal Biting Collections:

A total of 31 two-hour seasonal collections conducted from January-October 2002 generated a total of 2,010 *An. darlingi* (Table 2). Other anopheline species captured in decreasing densities include: 162 *An. albimanus*, 65 *An. punctimacula*, 30 *An. apicimacula*, 24 *An. vestitipennis*, 17 *An. pseudopunctipennis*, 11 *An. gabaldoni* and 2 *An. crucians*. In addition, 14 aberrant morpho-types of *An. darlingi* were also collected.

Three collections were performed each month except for the months of January (6 collections), May (1 collection) and July (6 collections). No collections were performed

in the months of June and November-December. The months of January (110), May (310) and July (99) exhibited the highest monthly average of biting *An. darlingi* females (Table 2). These represent transitional periods between the end of the wet season (January) and the end of the dry season (May-July). The three collections performed in April averaged the smallest number (15) of *An. darlingi* adults.

Overall, *An. darlingi* populations exhibited an exophagic biting behavior with a total of 923 collected indoors and 972 outside the experimental hut giving an indoor to outdoor ratio of 1.00:1.05 (Table 2). However upon monthly examination, an endophagic response was seen in six (February, March, April, August, September and October) of the nine months in which collections were conducted, with indoor to outdoor ratios ranging from 1.00:0.88 in March to 1.00:0.34 in August.

Annual rainfall for the year 2002 totaled 3,237 mm, with 83% (2,697 mm) falling in the wet season (June-December) and 17% (540 mm) within the dry season (January-May). For the months in which the study was conducted, the highest precipitation level (15 mm) occurred during the month of July and the lowest (0.07 mm) in April (Figure 8A). The average monthly river levels showed a peak of 2.12 m in August, and the lowest level (1.19 m) occurred in January (Figure 8B). The average high temperature during the collection months ranged from 29°C in January to 34°C in the month of May (Figure 8C). Average low temperatures ranged from 19°C in January to 23°C from May-September (Figure 8D). All monthly environmental data can be found in Table 3.

The environmental trends of 2002 were similar to those in 2001 (Figure 9A-C). A total of 2,461 mm rainfall was recorded for 2001 with 79% (1,938 mm) occurring within the rainy season and 21% (524 mm) within the dry season (Figure 9A). Monthly river

data showed levels in 2002 were higher in the early months of the wet season as compared to 2001, although the highest and lowest levels recorded were similar for both years (Figure 9B). An average low of 21°C and an average high of 30°C was observed in 2001, which is similar to that seen in the study period (Figure 9C).

Nonparametric bivariate analyses of monthly average environmental parameters with monthly average densities of *An. darlingi* indicate no significant associations. Similar results were seen when data were examined by individual collection nights (Table 3). However, there was a relatively substantial negative correlation ($r=-0.220$; $p=0.235$) between river level and the total number of females collected; therefore, further examinations were conducted to determine if a stronger association could be described. The mean difference in the river level for the 14 days prior to the date of a collection (i.e., oviposition to adult emergence with 24-hr. pre- host seeking) was calculated and correlated with *An. darlingi* densities for the individual collection (Figure 10). Nonparametric statistics consistently indicated a non-significant association between mean difference in height of river and populations captured ($r=-0.279$; $p=0.128$).

Seasonal trends of other anophelines collected at the study site were also examined (Figure 11). *Anopheles albimanus* had the highest population densities (57%; 93/162) during the months of July–October, and similarly, *An. vestitipennis* exhibited highest catches (22/24) during August–October. The majority of *An. pseudopunctipennis* (65%; 11/17) were captured in the month of April but adults were also collected August–October. *Anopheles punctimacula* females exhibited two peak population densities; one in January (21/65) and another in August (21/65). *Anopheles apicimacula* populations were highest during the months of January–April (28/30).

Natural Sporozoite Infections:

The Cayo District reported a total of 153 cases of malaria for the 2002 study year (Figure 12A). This was slightly less than the 184 reported in 2001 (Figure 12B). There were no cases of *P. falciparum* reported for either year from the Cayo District. Malaria case distributions, for those villages within a 5-mile circumference of the study site reporting cases (i.e., Armenia, Caves Branch and Hershey), reported 5 total cases of *P. vivax* for the year 2002 and 7 total cases of *P. vivax* in 2001 (MOH). In 2002, two cases occurred in February with one case occurring each in March, June and September (Figure 12A). In 2001, the majority (4/7) of cases were detected during the month of July, with one case each occurring in May, August and November (Figure 12B). The low number of local malaria cases prevented statistical analyses of associations between disease prevalence and *An. darlingi* monthly densities to be performed. However, descriptive analyses indicate a trend in high vector densities and the presence of local cases within the following months. At a district level, trends were also seen between increasing *An. darlingi* populations and a rise in malaria cases (Figure 13). When bivariate correlation analyses were performed, a negative association was indicated although not significant ($r=-0.370$; $p=0.327$). In order to detect natural infections, a total of 10,242 *An. darlingi*; 373 *An. albimanus*; 216 *An. pseudopunctipennis*; 88 *An. punctimacula*; 78 *An. vestitipennis*; 44 *An. apicimacula*; 14 *An. gabaldoni* and 4 *An. crucians* specimens from both the all-night and seasonal collections were screened for malaria sporozoites using the VecTestTM. No positive pools were detected.

DISCUSSION

It is important for workers in endemic countries to understand the role of specific anopheline vectors in disease transmission in order to implement successful, cost-effective control methods. This includes defining the nightly biting patterns, indoor/outdoor biting ratios and associations between seasonal population densities and malaria case distributions. Previous studies have incriminated *An. darlingi* as an important vector species throughout its geographic distribution. This is based upon natural sporozoite infections (Davis 1931; Arruda et al. 1986; Herrera et al. 1987; Oliveira-Ferreira et al. 1990), host-feeding preferences and indoor biting behavior (Deane et al. 1946; Roberts et al. 1987; Klein and Lima 1990; Rozendaal 1989; Grieco 2000), as well as positive associations between *An. darlingi* densities and peaks of regional malaria transmission (Ferraroni and Hayes 1979; Lourenco-de-Oliveira et al. 1989; Charlwood 1980).

For these reasons, *An. darlingi* has been the focus of several behavioral studies in Belize in order to understand its role in local malaria transmission. Kumm and Ram (1941) first described natural sporozoite infections in *An. darlingi*, and recent studies have also detected *P. falciparum* infections in wild-caught females collected from inside local homes (Achee et al. 2002). Within the laboratory, *An. darlingi* populations from Belize have been shown to have both a high salivary gland (41%) and midgut (53%) susceptibility to a laboratory strain of *P. falciparum* (Grieco et al. 2001). In addition, results from remote sensing and GIS studies have shown the highest malaria incidence in Belize occurs within villages located at a distance \leq 1-km of previously defined *An. darlingi* larval habitats (i.e., fresh-water rivers) (Hakre 2003). Despite these results, no

specific studies have previously been conducted to define the all-night indoor/outdoor biting patterns and ratios of *An. darlingi* until the present study. Likewise, this research was the first to describe the changes in *An. darlingi* densities by season and describe the relationship between population densities and local malaria distribution for the Cayo District of Belize.

Results in the present study show that *An. darlingi* populations exhibited a bimodal peak biting pattern. The strongest indoor and outdoor biting peak both occurred three hours post-sunset, however, relatively high numbers of females were found to bite throughout the entire night at both collection stations. Because no other 12-hr *An. darlingi* collection studies have been conducted in Belize, comparisons of populations within other regions of the country cannot be made. However, in studies from South America, *An. darlingi* has been shown to exhibit unimodal (Hudson 1984; Lourenco de Oliveira et al. 1989; Rozendaal 1990), bimodal (Forattini 1987; Tadei et al. 1988) and trimodal (Pajot et al. 1979) biting activity patterns. A study from Honduras has described a unimodal biting pattern for *An. darlingi* (Zimmerman and Rangel 1990). Despite changes in peak of activity, all studies described this vector to continue to bite during the entire night. Because of the inconsistency in biting patterns, the possibility of genetic diversity between Central American and South American populations of *An. darlingi* has been examined with results indicating there is no evidence for such a conclusion (Manguin et al. 1999). Interestingly, Voorham (2002) described differences in all-night biting patterns within one study site in Brazil over the course of six months; however, the majority of specimens were still collected during the nocturnal period. When examined independently, each collection night in the present study described similar activity

patterns of *An. darlingi*. The all-night activity pattern presented in the current study are similar to the activity pattern of *An. darlingi* reported from several studies in Brazil, where a major peak in biting occurred within three hours post-sunset and another minor peak occurred during the last two hours pre-sunrise with biting continuing throughout the night (Charlwood and Wilkes 1979; Charlwood and Alecrim 1988; Roberts et al. 1987; Klein and Lima 1990).

Described as an endophagic vector throughout South America (Giglioli 1948; Elliott 1972; Hudson 1984; Charlwood 1980; Fleming 1986; Roberts et al. 1987), Kumm and Ram (1941) were the first to document the occurrence of indoor *An. darlingi* biting populations within Belize. The present study also indicates an endophagic biting behavior of *An. darlingi*. The outdoor to indoor all-night biting ratio reported here, however, is much stronger ($O:I=1.00:1.04$) than that described for other *An. darlingi* populations located within the same region as the present study site ($O:I=1.00:0.6$) (Roberts et al. 2002). Another study, conducted in the Toledo District, also found *An. darlingi* to have a very weak endophagic behavior ($O:I=1.00:0.53$) (Grieco 2000). It should be noted however, that both of these studies only utilized data gathered from collections made during the first two hours post-sunset. Grieco (2000) emphasized the need to conduct all-night collections for describing biting behaviors when it was shown that *An. vestitipennis* populations exhibited a much stronger indoor biting activity from 12 hr ($O:I=1.00:0.90$) versus 2 hr ($O:I=1.00:0.53$) collections. Similar results have been shown in biting studies of *An. darlingi* in South America (Voorham 2002).

In addition to varying sampling periods, biting activity of vectors may also be affected by the lunar cycle in which the collection was performed (Bidlingmayer 1985).

Several studies have shown how moonlight influences both the density of biting populations and the time of peak capture (Charlwood et al. 1986; Elliott 1972). For this reason, the present data were examined for influences of moon phase on *An. darlingi* biting activity. There were significantly more total females collected both indoors and outside during full moon nights compared to other phases, and the cumulative majority of females on full moon nights were captured one hour earlier than those collections conducted during other lunar cycles. However, no differences were seen in the densities of *An. darlingi* captured during peak biting hours (i.e., three hours post-sunset) or during the nocturnal period (i.e., hours 4-10), and the peak biting time remained the same. Other studies in Columbia, South America found *An. darlingi* to show earlier peak biting during new and crescent moon phases compared to other lunar cycles (Elliott 1972). The differences were up to 100 minutes in the time required to complete 50% of the night's biting. Although the present study attempts to describe variations in *An. darlingi* biting activity according to moon phase, it should be noted that no illumination data were recorded; therefore, behavior patterns do not reflect light intensity influences as a result of either lunar cycle or time of moon rise and moon set. Such information would prove useful in future studies.

Because of their importance in malaria transmission in the coastal lowlands and foothills throughout the Central and South American region (Forattini 1962; Rodriguez and Loyola 1989; Ramsey et al. 1986; PAHO 1991), general descriptions of biting behaviors for both *An. albimanus* and *An. pseudopunctipennis* from the present study have been described. Considered throughout its geographic distribution to be exophagic (Bown et al. 1991; Rodriguez et al. 1991; Roberts et al. 1993), *An. albimanus* biting

populations collected over the night in the present research indicate a relatively strong endophagic response ($I:O=1.00:0.99$). Other all-night studies conducted in the Toledo District of Belize, have described *An. albimanus* to have a relatively strong exophagic biting behavior with outdoor to indoor ratios of $1.00:0.01$ (Grieco 2000). This discrepancy may be due to the geographic variation of study sites as well as the lower numbers of specimens collected in the latter study (158) compared to the present research (527). However, it should be noted that different strains of *An. albimanus* have been identified in Mexico according to larval morphology, and the behavioral plasticity may reflect genetic variations (Warren et al. 1979). Other 2- hr biting collections conducted at indigenous homes in the same region as the present study site have also shown an exophagic biting behavior in *An. albimanus* females ($O:I=1.00:0.21$; Roberts et al. 2002; Roberts et al. 1993). This difference is most likely due to the variation in the sampling periods (i.e., 12-hr versus 2-hr) in which collections occurred. However, other contributing factors could be the position of the doors and windows of the indigenous homes compared to the experimental hut used in the present study. Despite the differences in the biting ratios of *An. albimanus*, the bimodal peak in biting activity and the relatively low numbers of biting females throughout the night described here is similar to those patterns described for *An. albimanus* in other regions of Belize (Grieco 2000).

The biting behavior of *An. pseudopunctipennis* has been defined in limited areas throughout its geographic distribution (Fernandez-Salas 1992; Zimmerman 1992). Currently, no information is available for all-night biting patterns in Belize. As such, this study offers further insight into the behavior of this important vector. In the Tapachula

Foothills of southern Mexico, *An. pseudopunctipennis* was shown to exhibit an exophagic biting behavior with a bimodal activity pattern throughout the night (Fernandez-Salas 1992). Although the total number (371) of *An. pseudopunctipennis* collected during the study was relatively low, data from the current research indicate *An. pseudopunctipennis* to also have a bimodal biting pattern but more prominent endophagic behavior (I:O=1.00:0.53) than either *An. darlingi* (I:O=1.00:0.96) or *An. albimanus* (I:O=1.00:0.99). In addition, *An. pseudopunctipennis* exhibited a longer biting peak, with the cumulative majority not being captured until one hour later than the other two vector species. These results suggest an important role for *An. pseudopunctipennis* in malaria transmission in Belize and further studies focusing on this species are warranted.

The physiology of an insect is greatly influenced by the surrounding environment; therefore, the relationship between such parameters as temperature, humidity, rainfall and wind speed will affect vector-host contact (Elliot 1972). Understanding how these variables affect a vector's biting behavior is an important concept in malaria epidemiology. Results from the present study indicate that *An. darlingi* biting populations were only influenced (negatively) by wind speed for the collection night. Specifically, this relationship was only seen between the indoor populations. This may suggest influences upon the resting behavior of this species. If *An. darlingi* females rest out of doors for a time prior to entering a house to feed, then high wind speeds may prevent this behavior. Because there was a host positioned outside of the hut, outdoor feeding would not be affected as was seen in the analyses. Studies from South America have indicated such behavior patterns by describing outdoor resting during pre-sunset hours (Roberts et al. 1987), and upon entering a house, resting for only short periods of time before feeding

(Elliot 1972; Hudson 1984; Charlwood 1980). However, no systematic surveys have been conducted in Belize regarding the resting behavior of *An. darlingi*. Such studies are needed in order to understand relationships between environmental parameters and biting densities.

Descriptions of *An. darlingi* adult seasonal population distributions presented here are the first systematic report from the central Cayo District of Belize. Densities were highest during the months of January, May and July, all corresponding to the transition periods between the wet and dry seasons, with May exhibiting the highest density. The only other seasonal study of *An. darlingi* in Belize was from the southern Toledo District where *An. darlingi* exhibited largest adult populations during the dry season months of March and April, as well as the transition month of June, with the highest density (390/1020) also occurring in May (Grieco 2000). Roberts et al. (2002) found *An. darlingi* adults to be present at riverine locations within the region of the present study site during both the wet and dry seasons but only at upland sites (i.e., >1 km from rivers) during the wet season. However, these collections were only performed once at each house location and do not reflect longitudinal results. In South America, *An. darlingi* has shown high variability in seasonal distributions with some reports describing populations to be highest during the wet season (Rozendaal 1990), dry season (Rozendaal 1992) or correlated with transitional months between seasons (Ferraroni and Hayes 1979; Charlwood 1980). These differences reflect the geographical variation in larval breeding sites.

The present study also attempted to associate *An. darlingi* adult densities with environmental data during the collection months. Unfortunately, none of the variables

had significant associations with adult populations. Examination of correlations, however, did prove insightful. *Anopheles darlingi* densities were higher in months during or following low Sibun river levels (i.e. January-May). The inverse relationship between precipitation (i.e., river level) and *An. darlingi* populations has also been described from Brazil (Charlwood 1980). As described in previous studies in South America (Rozendaal 1992), low river levels most likely lead to the persistence of detritus mats (i.e., preferred *An. darlingi* larval habitats) within the river and a low probability of “flushing”. Similar results were seen in the Toledo District of Belize, where *An. darlingi* populations were negatively associated with river levels (Grieco 2000). In order to investigate this theory of larval habitat persistence during dry seasons, the mean difference in river level data during 14 days prior to the night of a collection (i.e., oviposition to emergence with 24 hr pre- host-seeking) was correlated with adult population densities. Again, there was not a significant association, but trends were seen between negative mean differences (i.e., decreasing river levels) and high collection nights.

The hydrology of the Sibun River and its tributaries has not been investigated in depth due to the dynamic characteristic of the river. River width and depth vary tremendously both within and between seasons as does the flow rate, especially along the mid-reaches of the river. In this river, heavy rains create “flash-floods” that could eliminate suitable larval habitats for this species. Interestingly, a 100-year flood occurred in June of 2002 when 1,205 mm of precipitation fell over the course of June 18th-26th (National Meteorological Service). An astonishing 580 mm was recorded at the research site on June 19th alone. The Sibun river level at the lower reaches rose from 1.94 m on June 20th to a maximum of 3.97 m on June 23rd. Despite this, the present study shows

July as one of three months with highest populations. Counting back 14 days from the first collection on July 15th, habitats within the river would have been established and contained eggs only five days from the last heavy rain on June 26th. This suggests several theories: 1) Breeding habitats are available immediately following a rise in river levels due to debris washed into the river from floods; 2) *An. darlingi* females lay their eggs in these detritus patches upon the first days of accumulation; and 3) larvae from existing habitats may survive some degree of flooding action. The latter theory has been preliminarily described for the Sibun River area (Manguin et al. 1996). In that study, observations showed detritus mats stranded on the banks of a lagoon could produce live anopheline larvae when they were replaced back into the water. It is not known what survivorship would exist within the main body of the river during a flood. Further studies into all of these theories would provide vital information into local transmission of malaria along river systems.

Seasonal distributions of other adult anopheline species at the research site were similar to those described for other regions of Belize. *Anopheles albimanus* was present during each of the collection months but was at highest densities during the wet season (i.e., July–October). Grieco (2000) also found high *An. albimanus* populations during the wet season months in the southern Toledo District. The preferred breeding habitat of this vector in Belize is marshes containing cyanobacterial mats (Rejmankova et al. 1993), however, larvae have also been found breeding in rivers (Chapter 5), road-side ditches and flooded fields (Grieco 2000). This flexibility in oviposition site selection will maintain populations throughout the year and will allow densities to increase following periods of heavy rain due to increased habitat availability. *Anopheles vestitipennis* adults

were also captured in highest numbers during the wet season months of July–October. Again, these results are similar to those described in studies from southern Belize (Grieco 2000). This species breeds predominately in tall dense macrophyte marshes (Rejmankova et al. 1998), but larvae have also been collected from flooded forests during the rainy season (Grieco 2000). Populations will decrease during the dry season due to reduction in available habitats. *Anopheles pseudopunctipennis* adult populations were the densest in the month of April. This species has been previously characterized to breed in sunlit pools containing filamentous algae along waterways (Rejmankova et al. 1993). The population increase described at the study site corresponds to the period of lowest recorded monthly precipitation (0.07 mm). A decrease in precipitation will cause river levels to decrease exposing rock pools, producing more larval habitats.

It should be noted that extrapolating associations between *An. darlingi* adult populations at the present research site and monthly reported case distributions was performed primarily for hypothesis building. The only way to truly determine this relationship would be to conduct concurrent systematic vector surveys with human case incidence studies. As is typical throughout the country, malaria cases in the study area are passively detected through the Vector Control Office at the hospital located in Belmopan. This results in only those people who are intolerant of symptoms reporting to the health center. Because the predominant parasite species in Belize is *P. vivax*, which usually does not carry with it severe symptoms, positive cases may develop a degree of tolerance to symptoms and therefore do not seek medical care. In addition, the cost, time and availability of transportation to the local hospital may prevent infected persons from traveling to provide blood smears. For these reasons, the five reported cases within

villages surrounding the study site most likely is an underestimate of the true prevalence within the immediate locale. Deane (1986) stated that this species could transmit disease at low populations. This is most likely due to its high susceptibility to *Plasmodium* parasites (Klein et al. 1991). Although statistical tests could not be performed due to the low case numbers, descriptive analyses indicate that an inverse temporal relationship exists between vector density at the research site and local malaria cases. Similar trends have been reported from studies conducted in South America (Tadei and Thatcher 2000; da Silva-Vasconcelos et al. 2002). Although speculation, this may be a result of the recruitment of an older vector population that has a higher probability of being infective due to multiple feeding sessions, as a result, a disease transmission cycle could be maintained even at low *An. darlingi* density levels.

The inability to detect natural sporozoite infections in pools of *An. darlingi* from the present study site was not a surprise. Studies were conducted at this location specifically for its productivity in the target species but just as importantly for its isolation from other competing human-hosts. There is confidence that the negative VecTest™ results presented in the current study are real. Ryan et al. (2002) evaluated the VecTest™ in 16 test centers worldwide and reported a 97.8% accuracy overall, with 92.0% sensitivity and 98.1% specificity compared to the standard circumsporozoite enzyme-linked immunosorbent assay (ELISA). More specifically, comparison of the two methods using pools (4,654) of *An. darlingi* collected in Guatemala, Peru, and Venezuela indicated a 98.8% accuracy for both *P. falciparum* and *P. vivax*VK210, and a 96.3% accuracy for *P. vivax*VK247 infection. Given that these results reflect several different

geographic regions and parasite strains there is a high probability that similar sensitivity and specificity values would be produced in Belize.

In conclusion, this is the first time the all-night biting activity pattern and 12-hr indoor/outdoor biting ratios have been described for *An. darlingi* in Belize. In addition, the present study is the first to report the seasonal distribution of *An. darlingi* for the central Cayo District. Because of the propensity of *An. darlingi* to bite indoors throughout the night in relatively high numbers, its role as an important malaria vector in Belize is furthered confirmed. This is also suggested in the trend between monthly local malaria case distributions and vector population densities. Future efforts in Belize should focus on advanced adult behavioral studies of *An. darlingi* including the time of house entering/exiting to determine the time period in which *An. darlingi* will remain in a house before and after searching for a bloodmeal. In addition, the affect of insecticide spraying on adult biting and movement patterns should be evaluated. Furthermore, village based epidemiological studies involving active case detection of malaria infections with concurrent systematic vector surveys are needed to provide detailed information on the microepidemiology of malaria transmission. Such information would describe definitive roles of individual anopheline species in disease transmission throughout the country, and enhance decision-making processes for vector control.

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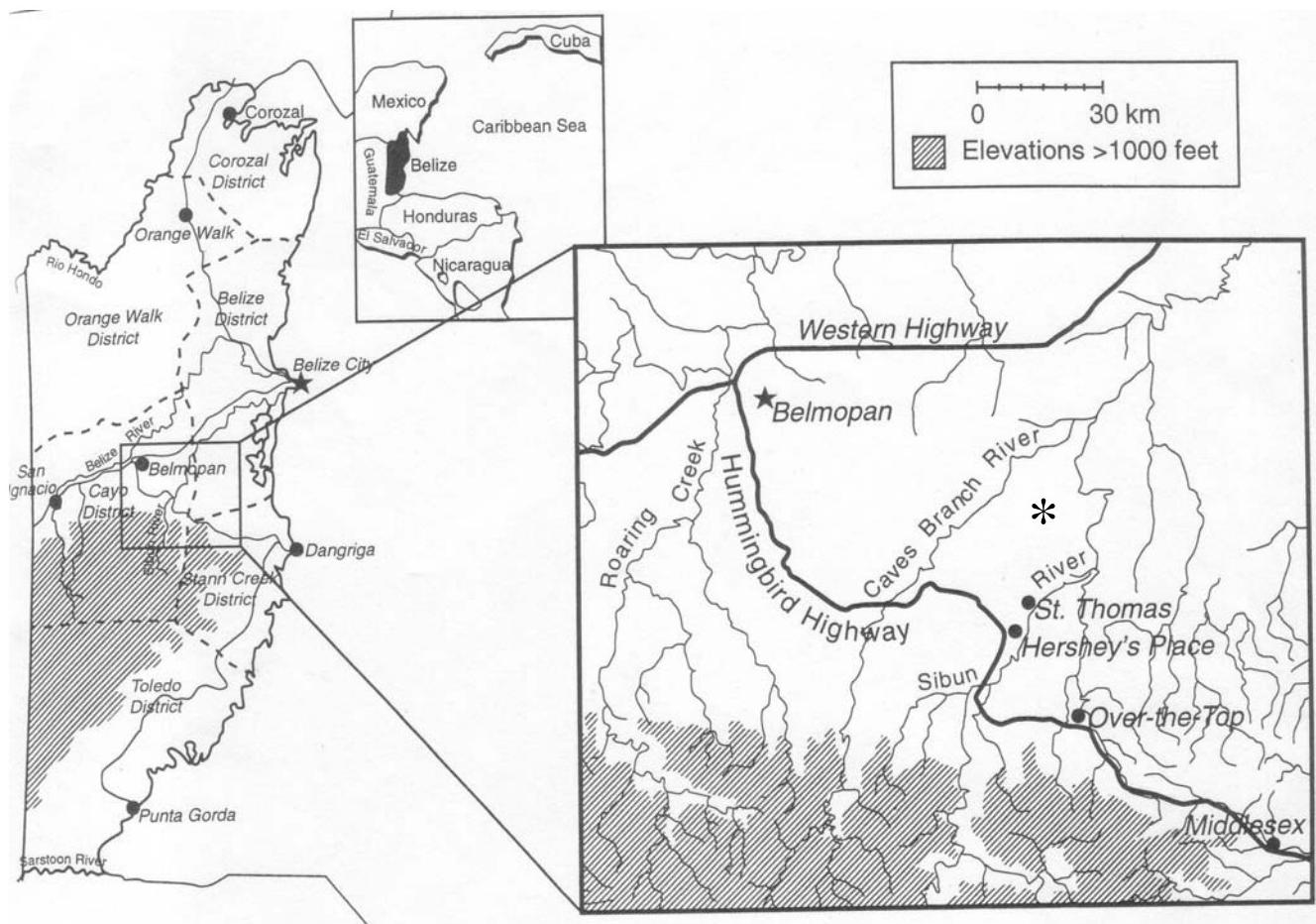


Figure 1. Map of the research site (*) within the centrally located Cayo District (Roberts et al. 1996).

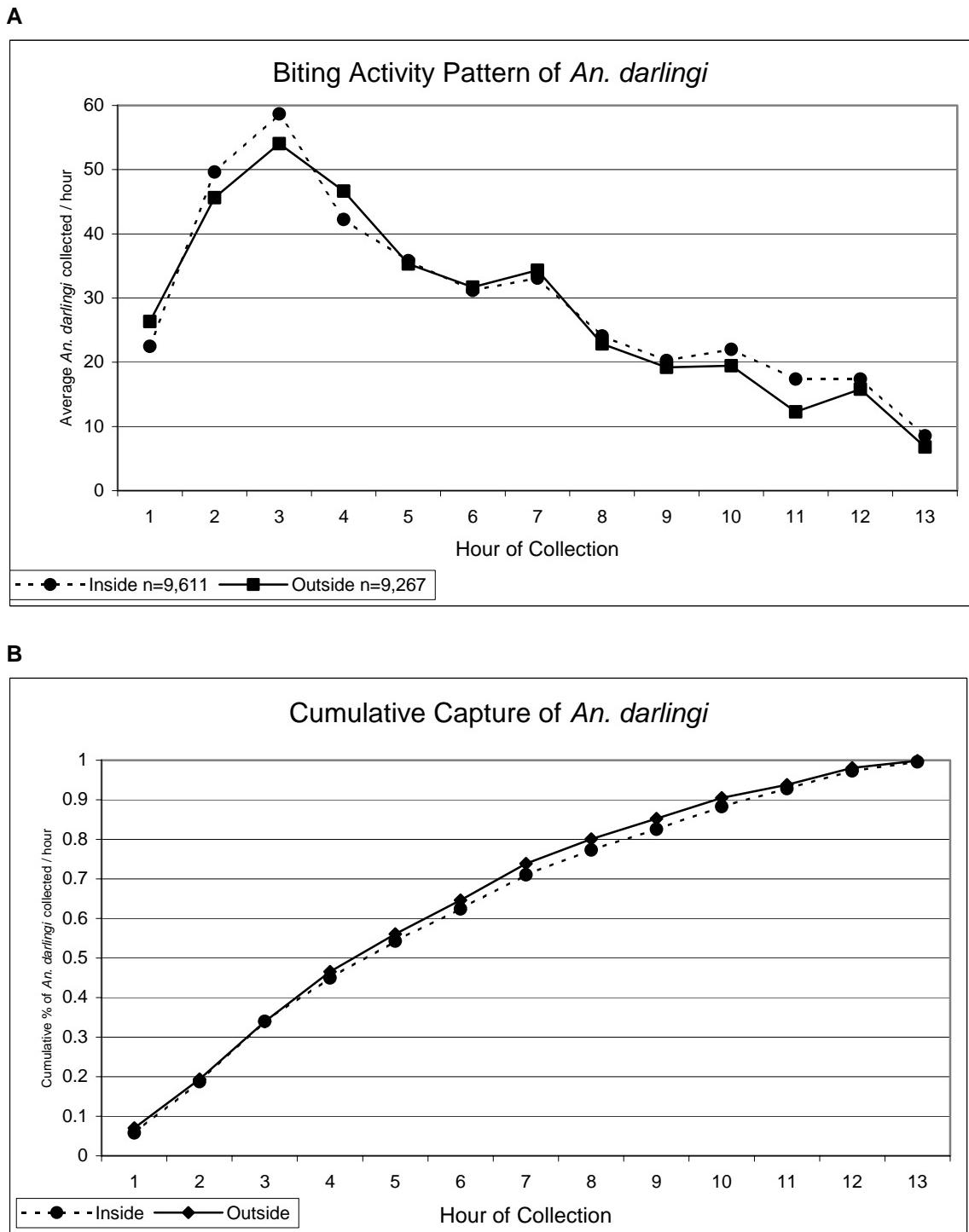


Figure 2. Graphs showing the nightly biting pattern (A) and cumulative capture (B) of indoor and outdoor biting *An. darlingi* (18,878) females captured during 25 all-night biting collections performed from January 2002-May 2003.

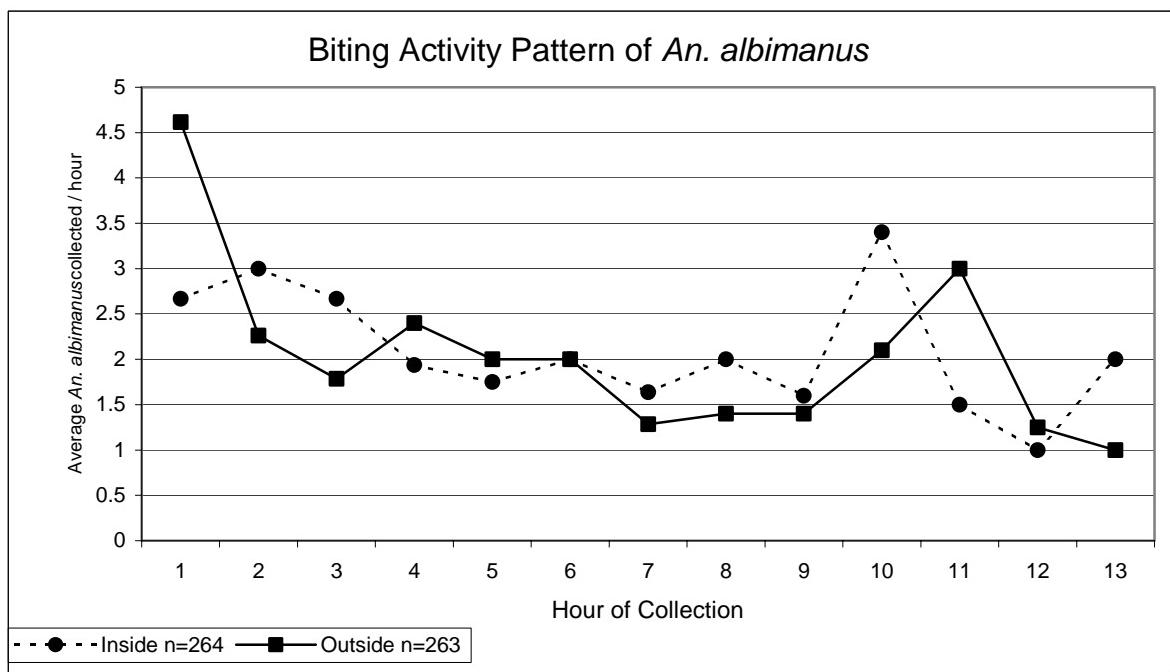
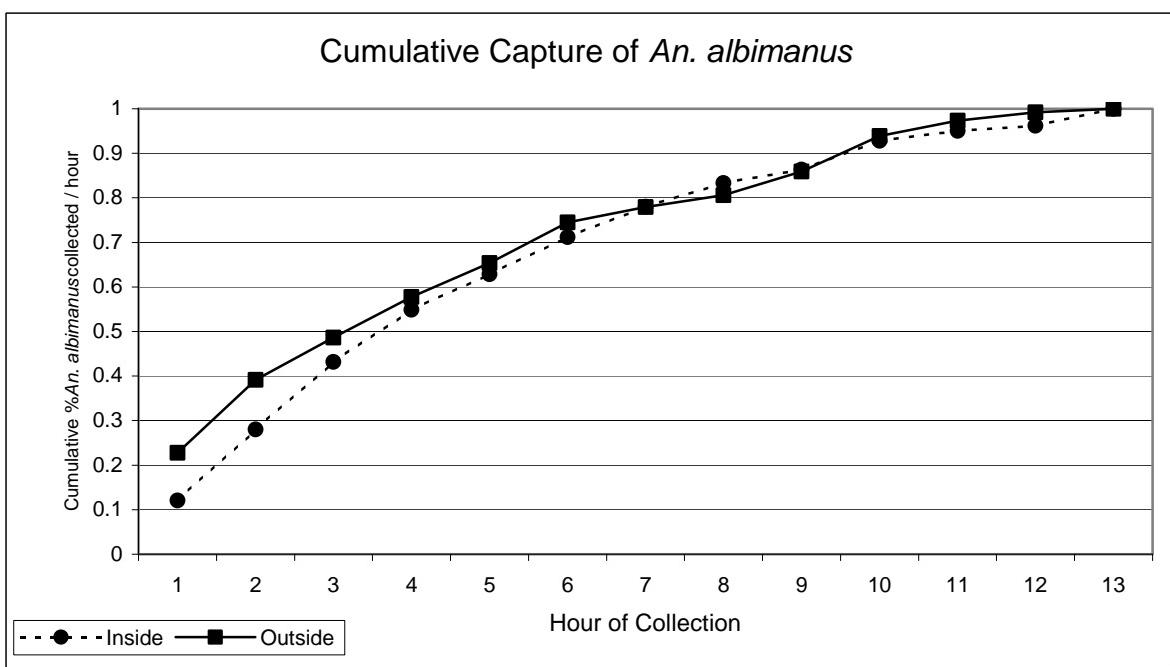
A**B**

Figure 3. Graphs showing the nightly biting pattern (A) and cumulative capture (B) of indoor and outdoor biting *An. albimanus* (527) females captured during 25 all-night biting collections performed from January 2002-May 2003.

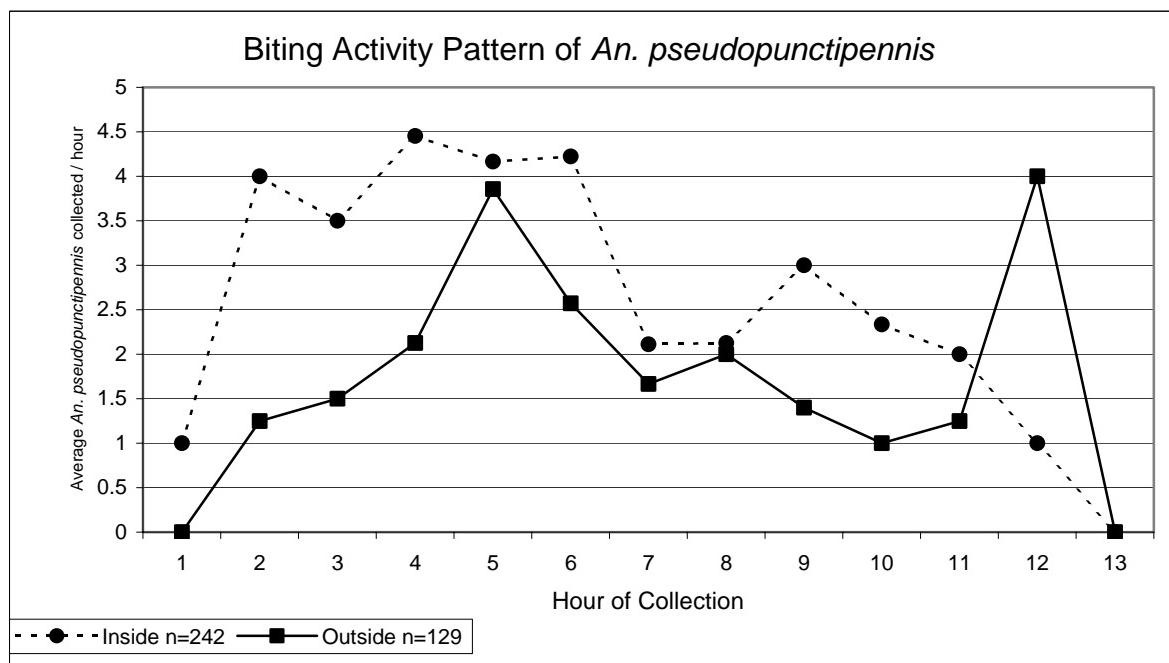
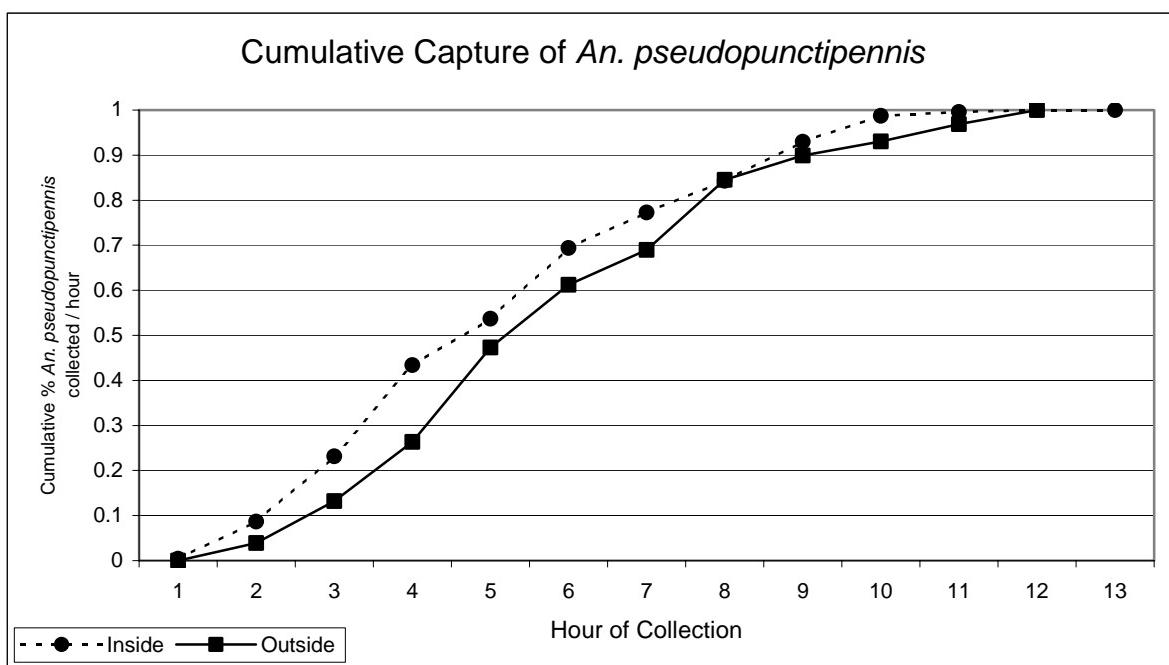
A**B**

Figure 4. Graphs showing the nightly biting pattern (A) and cumulative capture (B) of indoor and outdoor biting *An. pseudopunctipennis* (371) females captured during 25 all-night biting collections performed from January 2002-May 2003.

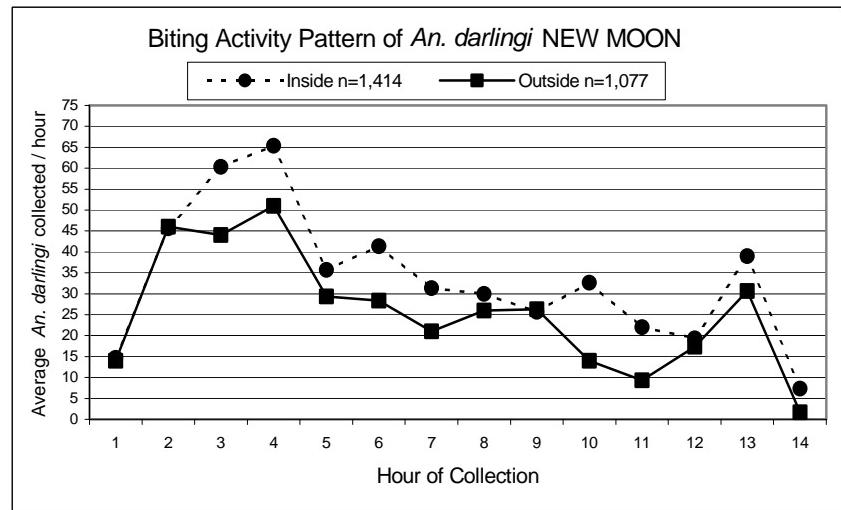
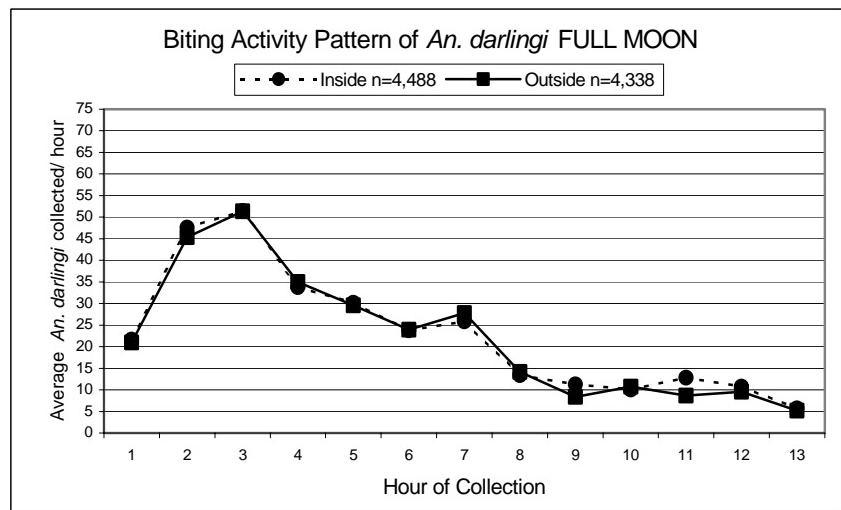
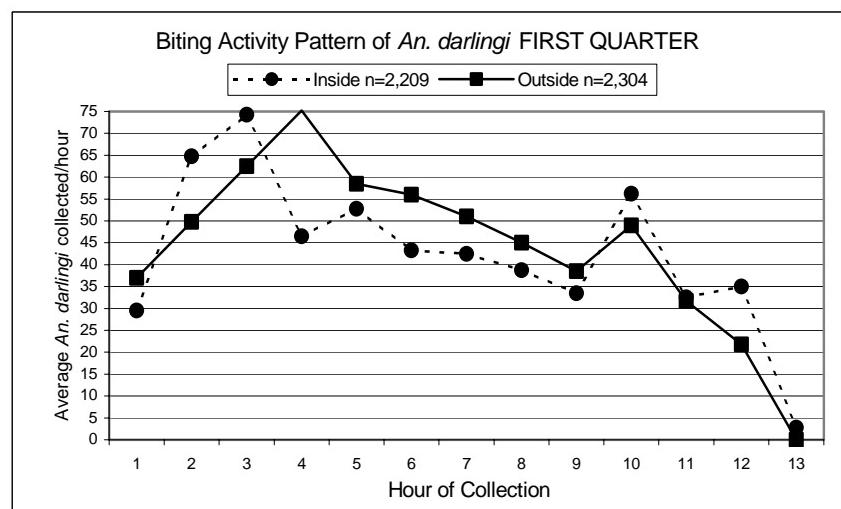
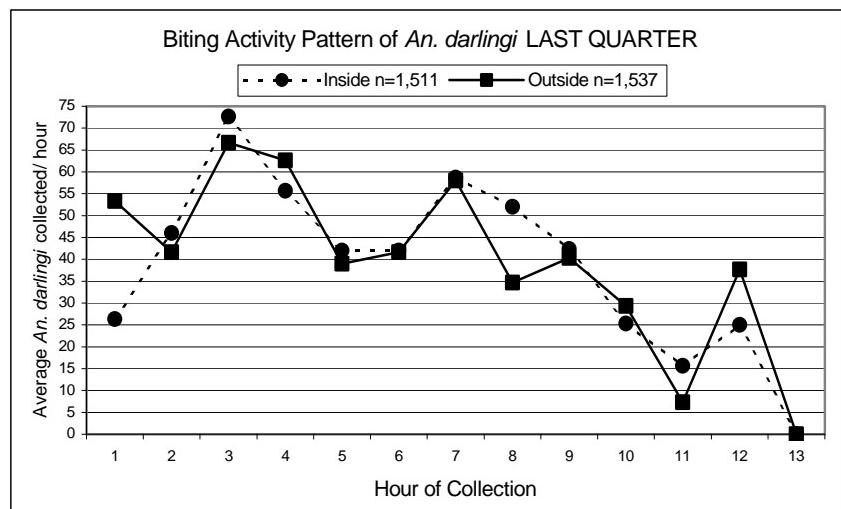
A**C****B****D**

Figure 5. Indoor and outdoor biting activity patterns of *An. darlingi* during each moon phase captured from 25 all-night collections conducted from January 2002–May 2003. (A) New Moon; n=3 trials. (B) First Quarter; n=4 trials. (C) Full Moon; n=15 trials. (D) Last Quarter; n=3 trials.

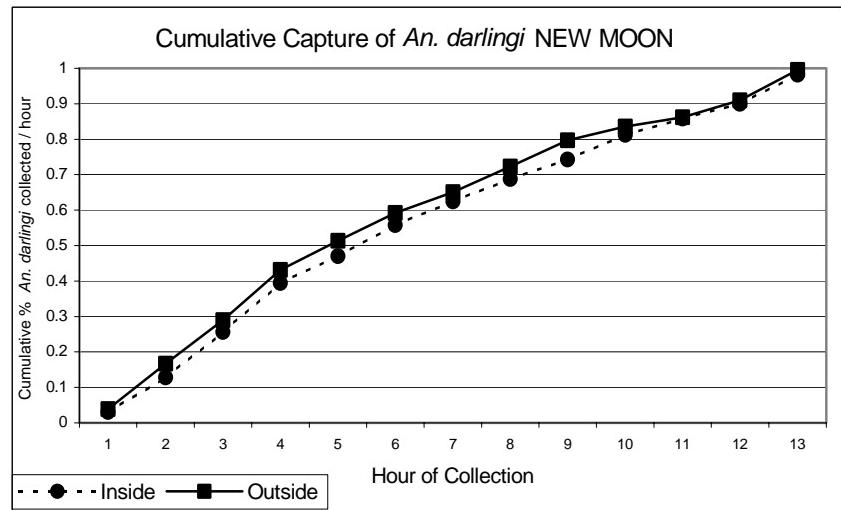
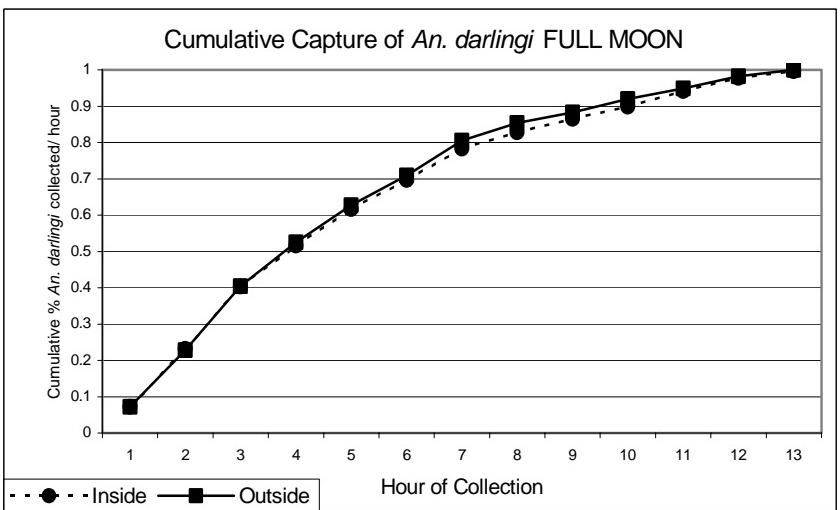
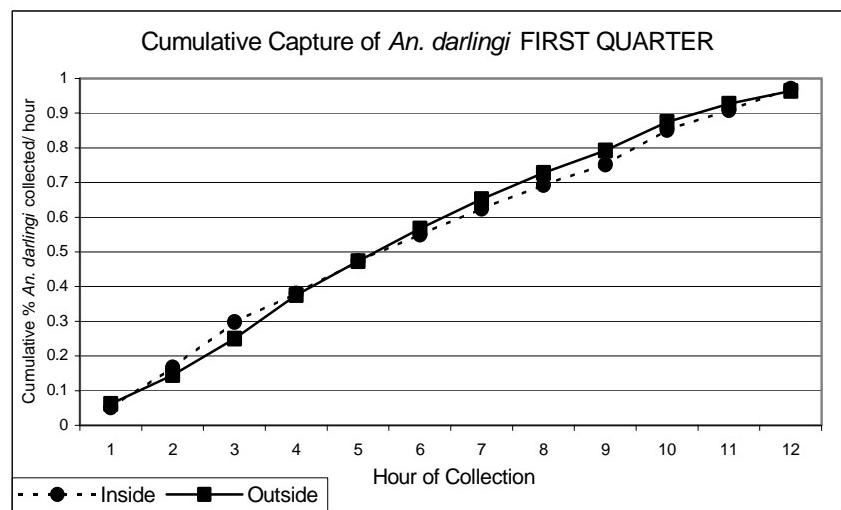
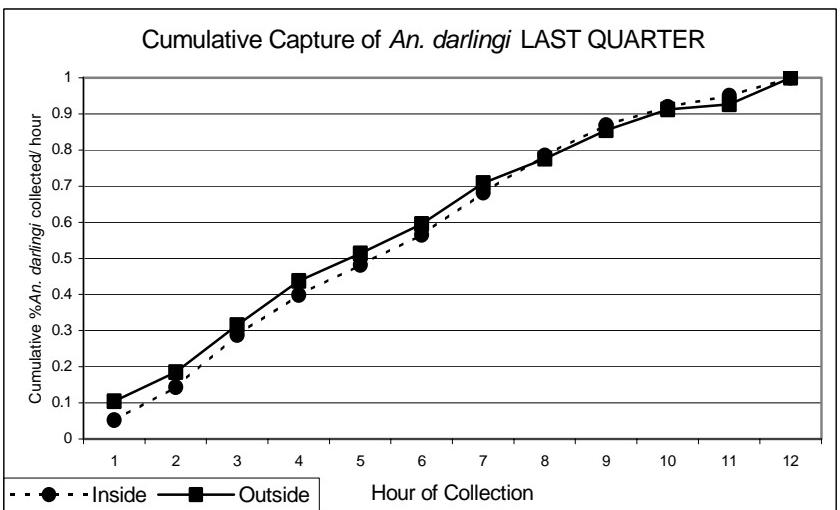
A**C****B****D**

Figure 6. Indoor and outdoor cumulative capture of *An. darlingi* during each moon phase captured from 25 all-night collections conducted from January 2002-May 2003. (A) New Moon; n=3 trials. (B) First Quarter; n=4 trials. (C) Full Moon; n=15 trials. (D) Last Quarter; n=3 trials.

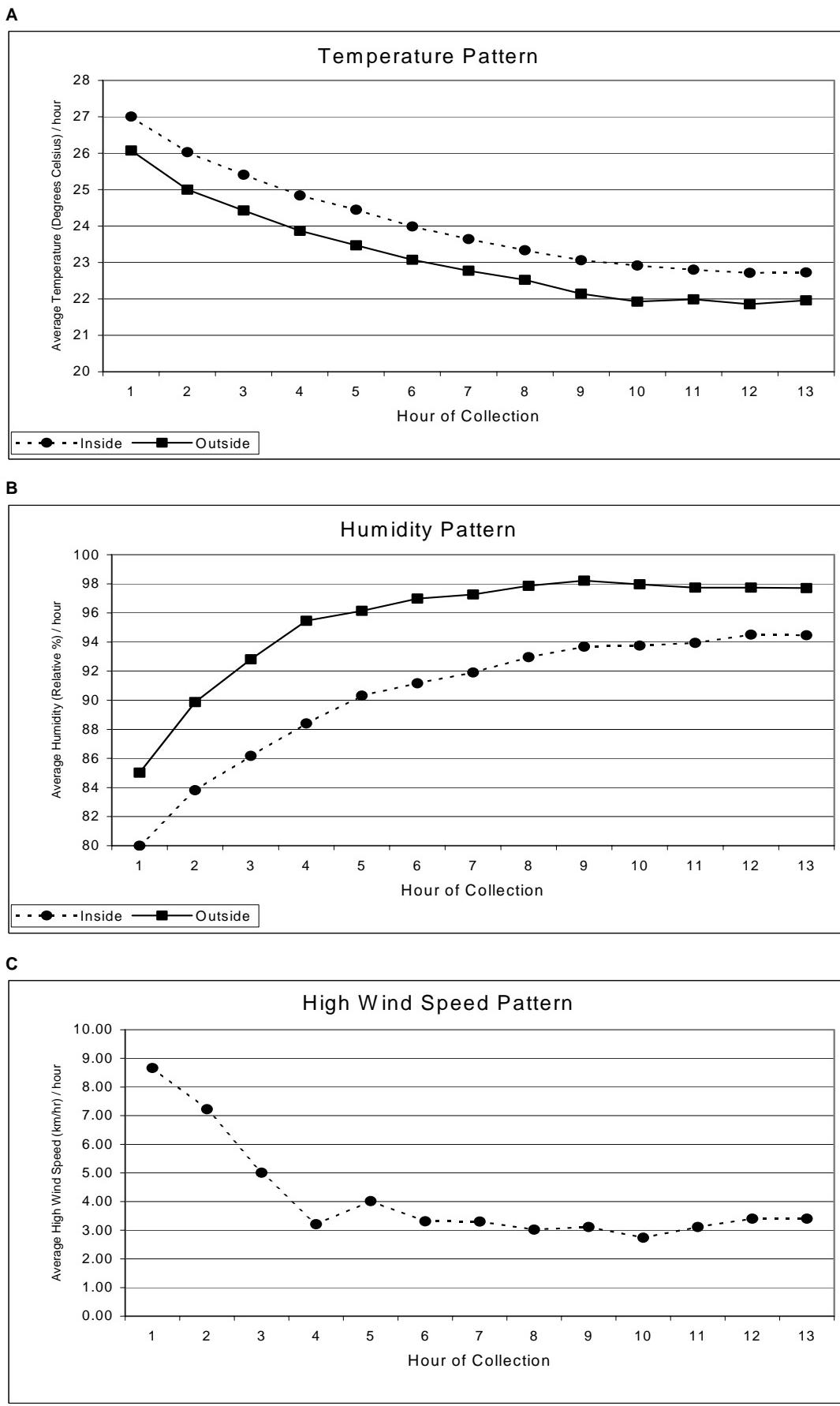


Figure 7. Graphs illustrating the average hourly weather patterns from 25 all-night biting collections performed from January 2002-May 2003 of (A) indoor and outside temperature, (B) indoor and outside humidity and (C) high wind speed for each collection hour.

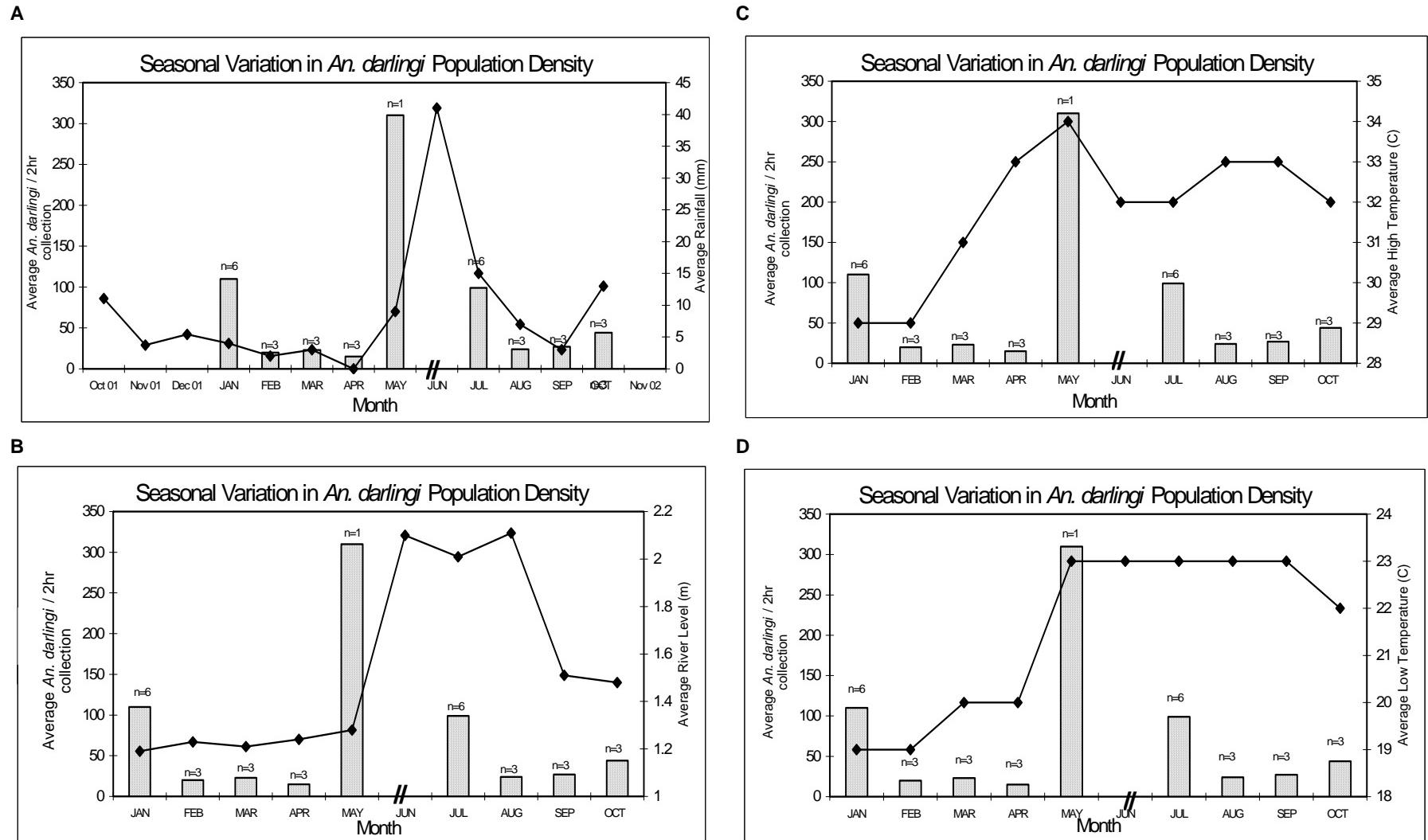


Figure 8. Graphs illustrating seasonal variation in *An. darlingi* population densities from a total of 31 two-hour collections performed during January-May and July-October 2002 with overlying environmental data including (A) monthly average rainfall; (B) monthly average river level; and both monthly average high (C) and low (D) temperatures. The number of collections performed each month are depicted above each bar.

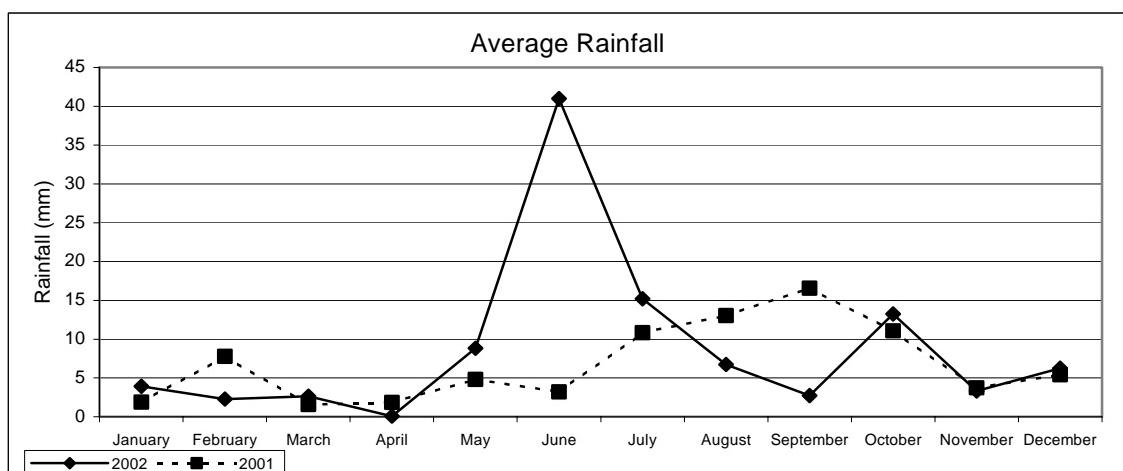
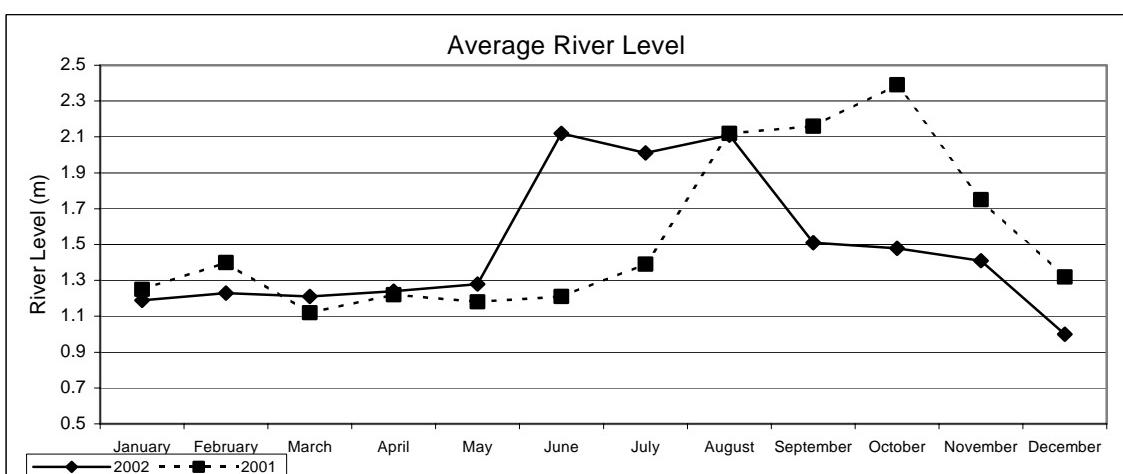
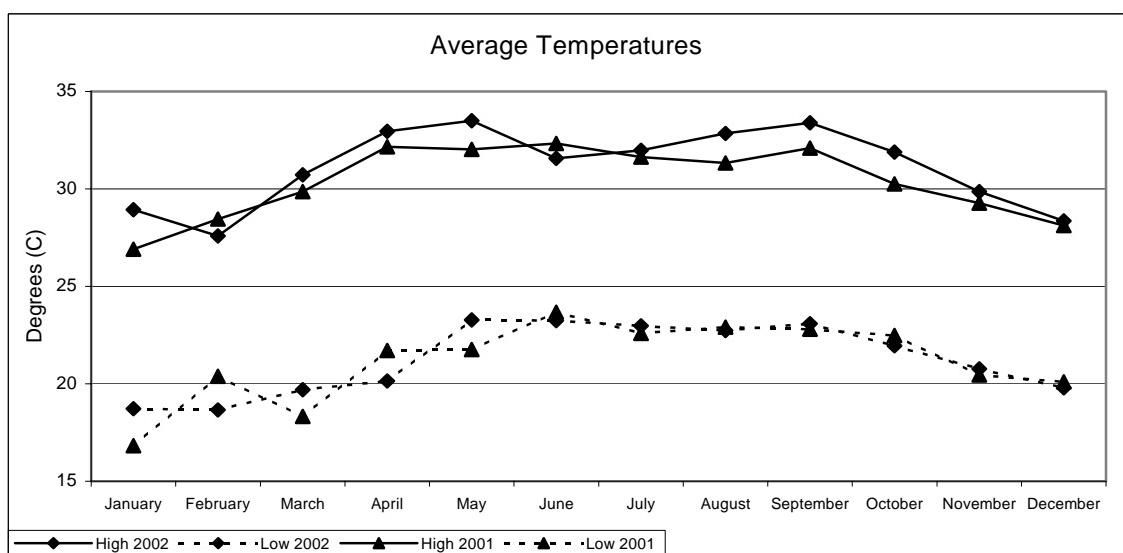
A**B****C**

Figure 9. Graphs illustrating monthly average environmental data for (A) precipitation, (B) Sibun River level and (C) high and low temperatures for the years 2001 and 2002.

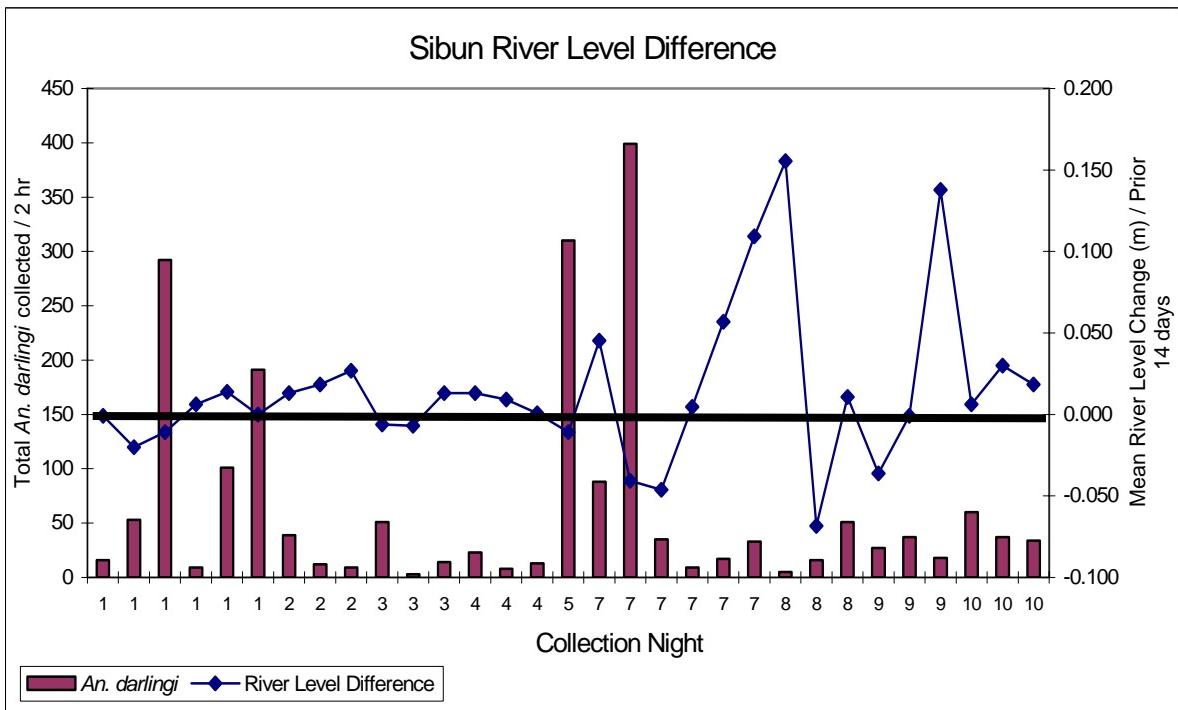


Figure 10. Total number of *An. darlingi* captured per night during 31 two-hour collections from January-October 2002, with overlay of mean river level difference for the 14 days prior to the collection. A negative difference represents a mean decrease in river level while a positive value represents a mean increase in the height of the river. Collection nights are represented according to the month performed (i.e., 1=January).

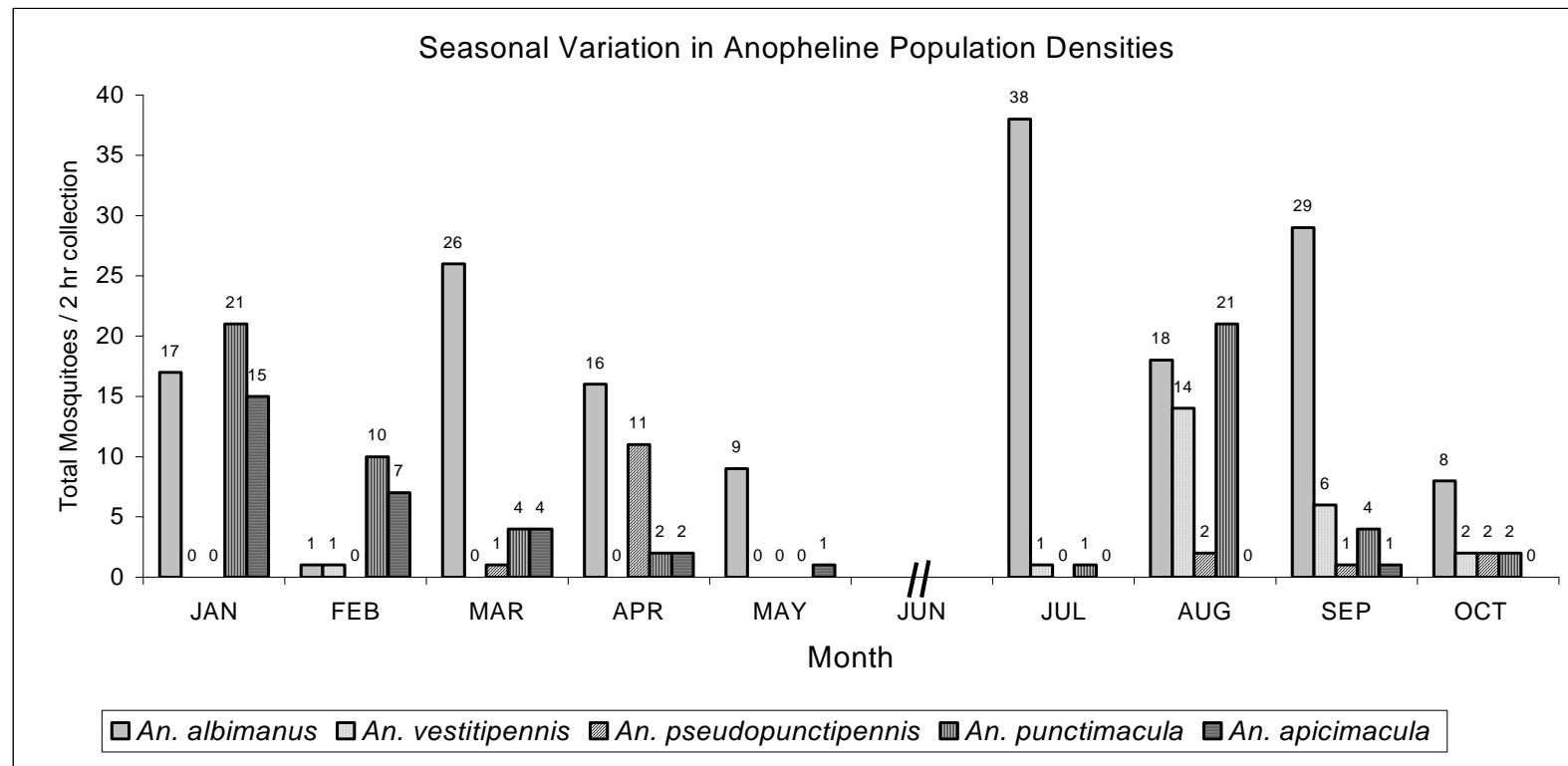


Figure 11. Graph illustrating seasonal variation in populations of other anophelines captured at the research site from a total of 31 two-hour collections performed during January-May and July-October 2002.

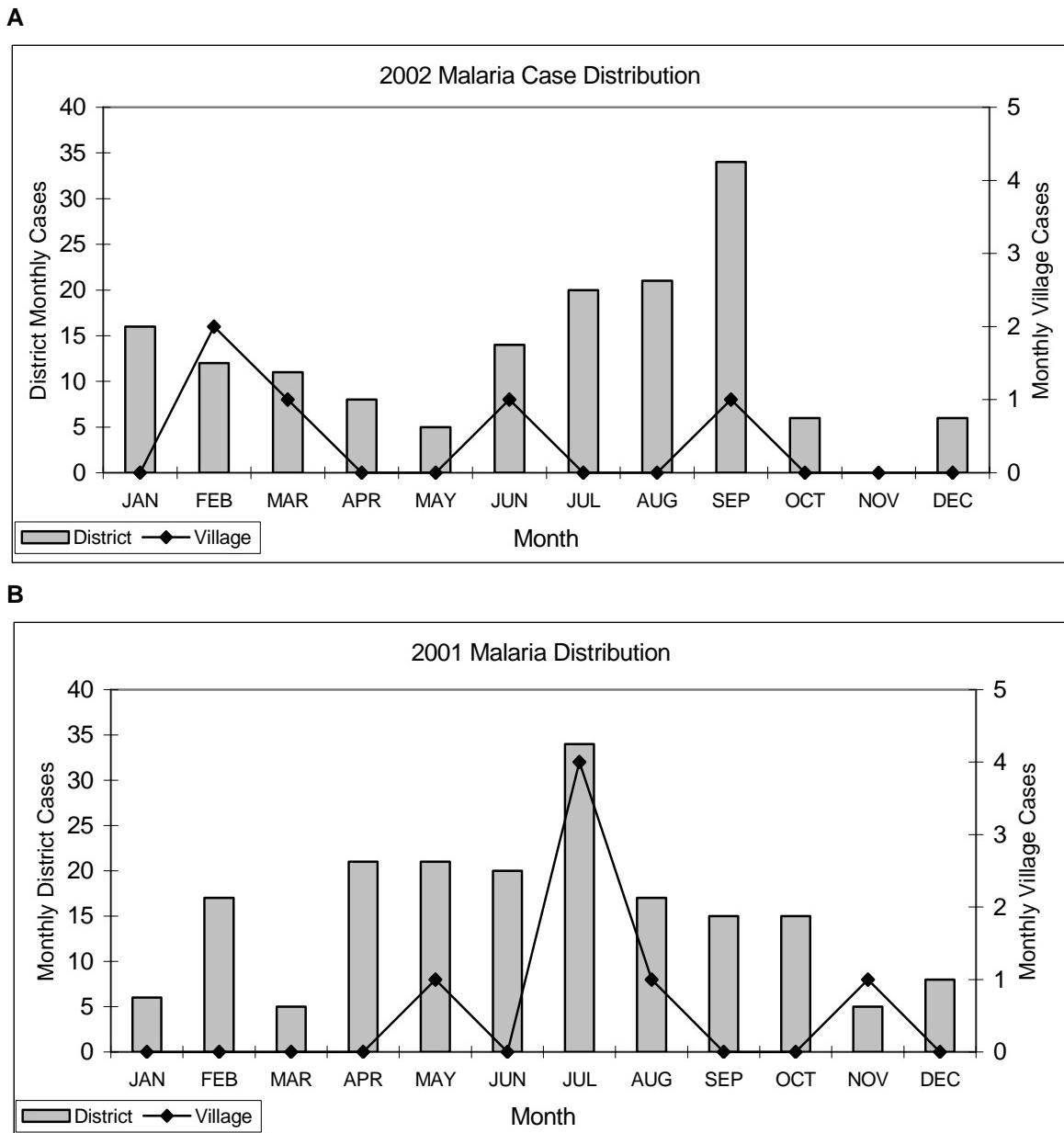


Figure 12. Monthly (A) 2001 and (B) 2002 malaria case distributions for the Cayo District and local case frequencies within villages in the immediate area of the research site (i.e., Armenia, Caves Branch and Hershey).

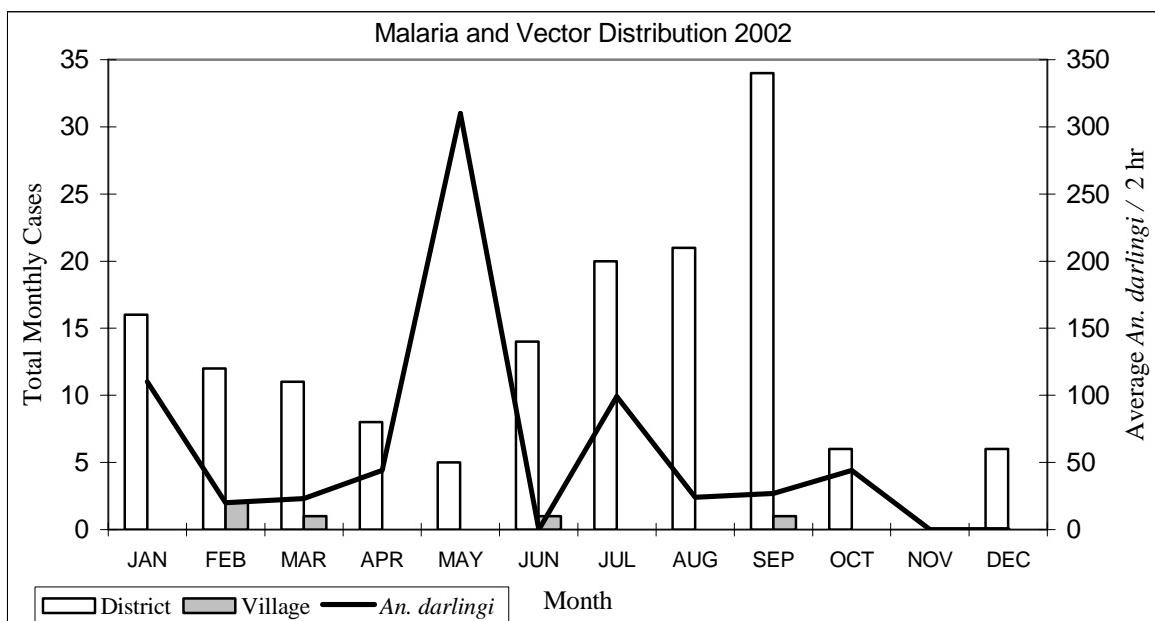


Figure 13. Monthly average number of *An. darlingi* captured during 2 hr collections overlaid onto district and local village malaria case distributions for the year 2002. Three collections were performed each month with the exception of six in January and July and one in May. No collections were performed in the months of June, November and December.

Table 1. Descriptive results of anopheline mosquitoes captured from 25 all-night biting collections conducted indoors and outside an experimental hut from January 2002-May 2003 as part of a study examining the biting activity pattern of *An. darlingi* in Belize, Central America

Species	Total Collected	Indoor	Outdoor	I:O
<i>An. darlingi</i>	18,878	9,611	9,267	1.00:0.96
<i>An. albimanus</i>	527	264	263	1.00:0.99
<i>An. pseudopunctipennis</i>	371	242	129	1.00:0.53
<i>An. punctimacula</i>	46	20	26	1.00:1.30
<i>An. vestitipennis</i>	44	24	20	1.00:0.83
<i>An. apicimacula</i>	21	10	11	1.00:1.10
<i>An. gabaldoni</i>	5	5	0	-
<i>An. punctipennis</i>	2	2	0	-
<i>An. crucians</i>	1	1	0	-
<i>Chagasia bathana</i>	2	1	1	-
Aberrant <i>An. darlingi</i> ^a	258	101	157	1.00:1.55

^aHarbach et al. 1993

Table 2. Results from 31 human-baited landing collections conducted indoors and outside an experimental hut for 2-hr. after sunset during January–October 2002. Total number of collections performed each month include: January=6; February=3; March=3; April=3; May=1; July=6; August=3; September=3; and October=3.

Species	January		February		March		April		May		July		August		September		October		I:O
	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	
<i>An. darlingi</i> n=2,010	293	369	36	24	36	32	28	16	118	192	236	345 ^b	38	34 ^c	45	37	93	38	1.00:1.05
<i>An. albimanus</i> n=162	5	12	1	0	14	12	9	7	4	5	6	32	12	6	12	17	4	4	1.00:1.42
<i>An. punctimacula</i> n=65	10	11	1	9	1	3	0	2	0	0	0	1	3	18	0	4	0	2	1.00:3.33
<i>An. apicimacula</i> n=30	6	9	4	3	0	4	0	2	0	1	0	0	0	0	1	0	0	0	1.00:1.72
<i>An. vestitipennis</i> n=24	0	0	1	0	0	0	0	0	0	1	0	7	7	4	2	2	0	1.00:0.60	
<i>An.</i> <i>pseudopunctipennis</i> n=17	0	0	0	0	0	1	2	9	0	0	0	0	0	2	0	1	0	2	1.00:7.50
<i>An. gabaldoni</i> n=11	1	0	0	0	1	0	0	0	0	0	0	0	0	4	0	1	0	4	1.00:4.50
<i>An. crucians</i> n=2	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	-
Aberrant <i>An. darlingi</i> ^a n=14	3	3	0	1	0	0	0	1	3	0	2	1	0	0	0	0	0	0	1.00:0.75
Monthly <i>An. darlingi</i> Avg.	110		20		23		15		310		99		24		27		43		
Monthly <i>An. darlingi</i> I:O	1.00:1.25		1.00:0.66		1.00:0.88		1.00:0.57		1.00:1.62		1.00:1.06		1.00:0.34		1.00:0.82		1.00:0.41		

^aHarbach et al. 1993.

^bFor I:O calculations 94 *An. darlingi* were removed from the total outside population because no indoor collections were simultaneously conducted during four sampling periods.

^cFor I:O calculations 21 *An. darlingi* were removed from the total outside population because no indoor collections were simultaneously conducted during two sampling periods.

Table 3. Environmental data for 31 two-hour *An. darlingi* adult collections performed from January-October 2002 in the Cayo District of Belize.

Month	Collection Date	<i>An. darlingi</i>	Average Daily Temperature (°C)	Daily Precipitation (mm)	Daily River Level (m)	Average Monthly High Temperature (°C)	Average Monthly Low Temperature (°C)	Average Monthly Precipitation (mm)	Average Monthly River Level (m)
January	1/15/02	16	25	5.5	0.95	29	19	4.0	1.19
	1/16/02	53	23	3.0	1.10				
	1/17/02	292	24	0	1.12				
	1/27/02	9	26	0	1.17				
	1/29/02	101	25	11.4	1.10				
	1/30/02	191	24	24.5	1.13				
February	2/25/02	39	23	0	1.37	29	19	2.0	1.23
	2/27/02	12	24	4.6	1.37				
	2/28/02	9	23	3.8	1.14				
March	3/19/02	51	33	0	1.19	31	20	3.0	1.21
	3/20/02	3	34	0	1.25				
	3/26/02	14	27	0	1.34				
April	4/15/02	23	26	0	1.15	33	20	0	1.24
	4/16/02	8	27	2.1	1.13				
	4/17/02	13	27	0	1.18				
May	5/7/02	310	28	0	1.24	34	23	9.0	1.28
July	7/15/02	88	29	0	1.58	32	23	15.0	2.01
	7/16/02	399	28	7.4	1.49				
	7/21/02	46	28	3.5	1.74				
	7/22/02	9	28	0	2.18				
	7/28/02	17	28	3.7	2.40				
	7/29/02	33	28	101	2.45				
August	8/5/02	5	28	0	2.71	33	23	7.0	2.11
	8/18/02	16	27	3.0	2.23				
	8/29/02	51	28	0	1.53				
September	9/3/02	27	28	0	2.0	33	23	3.0	1.51
	9/11/02	37	28	21.7	1.43				
	9/26/02	18	28	0	1.27				
October	10/3/02	60	29	0	1.56	32	22	13.0	1.48
	10/9/02	37	27	0	1.52				
	10/16/02	34	27	0	1.50				

Chapter 3

**A mark-release-recapture study utilizing a novel portable hut design to define the
flight behavior of *Anopheles darlingi* in Belize, Central America**

ABSTRACT

Knowledge of the flight behavior of local vectors is of paramount importance in mosquito control programs. The following study defined the recapture rate of wild-caught, unengorged *Anopheles darlingi* females at 0 M, 400 M and 800 M from a fixed release point in Belize, Central America, using a portable experimental hut.

Three sampling trials, each consisting of two 12-hr collections, were performed at all three distances from July 2002-June 2003. A total of 1,185 resting *An. darlingi* were marked and released during the course of the study. The recapture rate was greatest at 0 M (29.0%; 124/428) and was 11.6% (37/317.7) at 400 M, and 5.82% (21/361) at the 800 M site. There was no significant difference between the average numbers of marked mosquitoes recaptured inside versus those recaptured outside (0 M=8.6; 400 M=3.2; and 800 M=1.5) the experimental hut at each distance location.

Recapture rates of each trial were highest during the first night's collection at all distance locations. Further examination of the first night data revealed a variation in the peak time of recapture among distances. The peak in the total night's recapture at both the 0 M and 400 M sites occurred within two hours post-sunset, while the peak recapture at the 800 M site occurred during the seventh hour post-sunset.

Parity rates of the individual release populations were determined for each sampling trial. A total of 459 females were dissected with 65% (299) defined as nulliparous, 29% (134) parous and 5.7% (26) unidentifiable. There was no significant difference in the parity rates of the release populations among all distance locations. Examination of environmental variables indicated a negative correlation between the

numbers of marked *An. darlingi* recaptured and wind speed. No significant effect was described for indoor/outdoor temperature or humidity.

Information from the present study is the first to describe the flight behavior of *An. darlingi* in Belize. This data should prove beneficial in the development of adult density risk assessments within villages based on distances from potential vector breeding sites.

INTRODUCTION

Understanding the bionomics of a mosquito species is necessary in the monitoring and prevention of malaria. Research efforts in Belize have focused on four anopheline species that have been incriminated in the transmission of malaria including: *An. albimanus* Weidemann, *An. darlingi* Root, *An. pseudopunctipennis* Theobald, and *An. vestitipennis* Dyar & Knab. While all of these species have been shown to be competent vectors in the transmission of malaria in Central America and particularly Belize (Loyola et al. 1991, Padilla et al. 1992, Ramsey et al. 1994, Bangs 1999, Achee et al. 2000, Grieco et al. 2000, Grieco 2001), *An. darlingi* is presently considered one of the most important. This consideration is based on *An. darlingi*'s characteristics of being anthropophilic, exhibiting an endophagic feeding behavior, and its natural malaria infectivity rates (Kumm and Ram 1941; Deane et al. 1946; Foote and Cook 1959; Arruda et al. 1986; Achee et al. 2000).

Despite the medical importance of *An. darlingi* in malaria transmission, this vector's bionomics has been studied very little in Belize (Manguin et al. 1996, Harbach et al. 1993, Rejmankova et al. 2000, Achee et al. 2000, Grieco 2001), and to date, flight behavior research of *An. darlingi* has not been performed. Because the presence and

abundance of a vector species will contribute to its importance in disease transmission (i.e., vector-host contact), information on the host-seeking densities of a species at various distances from a potential larval habitat is vital in malaria studies. While research examining the flight behavior of several disease vectors exists, no systematic studies have been performed to date that determine the variability in flight behavior of anophelines using a portable experimental hut (Service 1993).

Mark-release-recapture studies are the sampling methods of choice in determining adult population attributes including flight distance capabilities (Service 1993). There are several marking methods including stains, paints, morphological modifications and radionuclides, but the use of fluorescent powders is presently the most common. Adult females are captured through human-baited, light trap or resting collections, dusted with the colored powder then later identified through the use of a ultra-violet light. Such methods have previously been used to examine the gonotrophic cycle (i.e. bloodmeal-oviposition interval) (Roberts et al. 1983) and resting behavior (Roberts et al. 1987) of *An. darlingi* in Brazil.

Amazingly, the literature is extremely sparse with information regarding the flight behavior of *An. darlingi* despite its role in disease transmission. Much of this is a result of this species' characteristic evanescent behavior (Hudson 1984), but very important information has come from the few studies performed. Charlwood and Alecrim (1989) described *An. darlingi* recapture rates of 12-19% in a study conducted in Rondonia, Brazil. These rates were much higher than the 2.3% reported for the other nine species of anophelines at the study site, further supporting the anthropophagic behavior of *An. darlingi*. In addition, two marked *An. darlingi* females were recaptured on the ninth day

post-release 7.2 km from the release site. The only other report from Brazil described a flight range of up to 2 km for this important vector (Deane et al. 1948). Such information is vital for defining spatial patterning of malaria transmission and developing risk assessments based on vector densities (Carter et al. 2000).

The objectives of the present study were to determine the recapture rates of *An. darlingi* indoors and outside a novel portable experimental hut stationed at various distances from a fixed release point. It is hoped this information will prove useful in developing risk assessments of adult vector densities at the house level and provide further insight into the role of *An. darlingi* in the transmission of malaria within the region.

MATERIALS AND METHODS

Study Site: The study site was established on open pastureland at 17°09'59.5" N 88°36'09.6" W in the central Cayo District of Belize, Central America (Figure 1). A freshwater system, the Sibun River flowed adjacent to the study site from which *An. darlingi* breeding habitats were previously located. The land is owned by Mr. Ramon Galvez Sr., whose house was the only permanent structure on the property. Domestic animals consisted of cattle (100), pigs (7), dogs (5) and horses (3). Temperatures ranged from 29°C in January to 34°C in May. Annual rainfall for the study year totaled 3,237 mm with 83% (2,697 mm) falling in the wet season (June-December) and 17% (540 mm) within the dry season (January-May).

Experimental Huts: Prior to conducting the study, a house survey was conducted in the village of Armenia, the closest village to the study site, to assure comparability (i.e., sleeping area and construction materials) of the experimental hut to local homes

(Appendix II). Two experimental huts were then constructed according to indigenous house design using locally acquired materials. Each 13 ft. x 13 ft. x 9 ft. hut had a roof made from corrugated zinc, walls and gables of untreated 1/2" x 8" wood planks and a dirt floor. Aluminum fence pipe (1 5/8" diameter) and galvanized pipe (1 3/4" diameter) were used for the framework (Figure 2A-E).

The huts were designed for portability by allowing the infrastructure, roof, gables and walls to collapse. The infrastructure collapsed by unbolting the twenty-6 ft. long aluminum fence pipe pieces from the eleven permanently welded galvanized pipe joints (i.e. 4 corner, 4 center and 3 roof pieces; Figure 2A). The roof collapsed into four hinged units each containing two-8 ft. long corrugated 25 gauge zing panels (Figure 2B). Holes were drilled in the lower corners of each panel such that each roof unit could be attached to the eave fence pipe for stability using wire. The gables were closed using 4 separate panels (Figure 2C). Each gable panel was attached to a 4" x 8" wood beam permanently joined to the front and back wall center pipe joints (Figure 2A). The walls were sectioned into 16 individual wood plank panels (Figure 2D). Four panels completed one side of the hut and were suspended from fence pipe pieces using 2" PVC hooks. Three of the wall panels contained a window measuring 2 ft. x 2 ft. and one wall board was a hinged door measuring 3 ft. x 6 ft. which attached to the 4" x 8" wood beam on the front wall center pipe using hinges.

Assembly took an average of 2 hours and disassembly 1 hour for four workers. The hut not in use was collapsed to prevent potential obstruction of host-seeking marked specimens. Huts were moved to pre-designated distances using a trailer hitched to a field vehicle. During recapture collections, both huts were set up at the study site. One hut was

completely assembled at the distance from which capturing was being performed (Figure 3), while the second hut remained at the 0 M site with only the pipe infrastructure in place. The wall, gable and roof panels for this hut remained on the trailer. When collections were performed at the 0 M distance, the wall, gable and roof panels were put in place and only the pipe infrastructure of the unused hut was standing. This design allowed for a reduction in labor by only disassembling one hut when rotating between distances. In addition, the partial break down of the unused hut was performed to increase the probability of recapture by preventing potential attraction of marked females to a second standing structure.

Distance Determination: Distance settings were based on quartile values (i.e., closest, 75% and furthest) of houses to a river margin (i.e., *An. darlingi* larval habitat). Values were determined by mapping individual homes within the villages of San Roman (Figure 4 and Appendix IV) and San Estevan (Appendix V) in the Orange Walk District. The villages were selected on the criteria of being adjacent to a river system and containing adult *An. darlingi* populations (Appendix I). In addition, both of these villages were apparent on high-resolution satellite imagery that permitted the visibility of both house and river locations allowing for accurate distance measurements. Villages within the Cayo District were not used because of this limitation.

Spatial locations of houses and the adjacent Rio Hondo River in San Roman were determined using hand-held GPS units in the field (Garmin International Inc., Olathe KS) and MapsourceTM v.3.02 computer software (Garmin International Inc., Olathe KS). Spatial locations of structures in San Estevan and the adjacent New River were

determined using an IKONOS satellite image in ArcView® GIS v.3.20 computer software (ESRI Inc., Reston VA).

Upon completion of mapping, distances were set at 0-meter (closest home), 400-meters (75% of homes) and 800-meters (furthest homes). Buffer zones of the same distances were set using the release point as the center to ensure the elimination of competing hosts at each interval. The release point was fixed at the edge of a tree line adjacent to the 0-meter site (Figure 1).

Resting Mosquito Collections and Marking: Resting, unengorged *An. darlingi* females were collected from shrub vegetation, a suspended tarp or from the outside mesh of a human-baited trap using a manual aspirator and flashlight. Collections began 30 min. prior to sunset and stopped when at least 100 females were captured or 3 hours had passed. No less than 50 females were released during any sample period. Specimens determined to contain a bloodmeal by visual inspection were not used in the study.

Captured mosquitoes were placed into two 1-gallon cardboard containers topped with mesh netting. One container was used for the release population and the other contained a control population used to determine mortality rates of the released population. As many as 20 females, but no less than 5, were used as a control population for each distance trial. Both populations were immediately marked after the collection with luminous marking powder (BioQuip Products, Inc., Gardena CA.) using a 1/4" paintbrush. The paintbrush was loaded with powder then quickly brushed against the mesh netting of the container lid in a circular motion from the outside circumference to the inside center of the container. This was repeated four times for each container. A paper towel moistened with distilled water was placed onto both containers, and the

mosquitoes remained in a humidified chamber (i.e., 25-gallon cooler) until time of release. Temperatures within the humidified chamber ranged from 14° C to 29° C, with an average of 19° C. Humidity ranged from 48% to 98%, with an average of 77%.

At 5:00 pm the following day, the number of dead mosquitoes within the release population container was recorded, and then the specimens were set free at a release site adjacent to the 0 M site using a time-release mechanism. Mortality rates for each release population were determined before the beginning of a recapture collection using the corresponding control specimens. This allowed for the determination of the total number of available marked specimens for each corresponding night. Immediately upon releasing the marked population, cotton pads containing a 10% sugar solution were placed on the top netting of the control populations. Sugar pads were moistened as needed until the end of the individual sampling trials. The color of marking powder was rotated for each sampling period (i.e., two collection nights) in order to distinguish the recapture of specimens from prior release nights.

Parity Rate Determination: An additional *An. darlingi* female population was captured for parity rate determination on the night of each resting collection. This population was captured using the same techniques as above and ranged from 40-60 females. The number of females varied from 40-60 and was determined by calculating the percentage of mosquitoes needed to ensure a 95% confidence interval of the parity rate of the release population with a 10% error. Mosquitoes were placed into two pint sized cardboard containers and killed immediately by placing an acetone-soaked cotton ball on top of each container and placing them into a closed 2.5-gallon cooler for 15 minutes. Females were identified to species (Wilkerson and Strickman 1990), and the ovaries were

dissected in 10% saline solution as previously described (WHO 1975). Slides were then kept in a sealed container with silica gel to dry until examination two days later using a compound microscope. Females were identified as nulliparous when coiled skeins in the fine tracheoles covering the ovaries were observed (Detinova 1945).

Recapture Collections: On the night of each release, two consecutive human-baited landing collections were used to recapture marked *An. darlingi* females. Collections started 30 min. prior to sunset and continued until 30 min. after sunrise. One collector was located approximately 2-m outside the hut while another collector was located at the center of the inside of the hut. All anopheline females landing on the exposed lower legs of the collectors were captured using manual aspirators and flashlights during a twenty-minute sampling period each half-hour. Collectors rotated positions (i.e., inside/outside) after each sampling period and were relieved with another set of two collectors after three hours. Mosquitoes were placed into modified cardboard ice cream pint cartons and killed at the end of each hour using acetone vapors within a killing chamber (i.e. a 1-gallon cooler).

Each carton was labeled with the collection hour (i.e., 1-13) and capture location (i.e., indoor/outdoor). Upon completion of the 12-hr collection period, specimens were poured into petri dishes, sorted by species (Wilkerson and Strickman 1990) and counted. Petri dishes were then placed into a ultra-violet light reading chamber to identify marked mosquitoes. Afterwards, specimens were placed in 1.5 ml Eppendorf vials properly labeled with date, indoor/outdoor station, species and number and then were placed over silica gel in a sealed container. The numbers of each anopheline species and marked *An.*

darlingi captured hourly at both the indoor and outdoor locations were recorded onto a corresponding form (Appendix VI).

Chi-square and ANOVA statistical analyses were performed to determine differences between parity rates and indoor/outdoor recapture rates by day of collection for each distance. The effect of environmental variables, including temperature (indoor/outdoor), relativity humidity (indoor/outdoor) (gathered using HOBO® Pro Series Weatherproof Data Loggers (Forestry Suppliers Inc., Jackson, MS)) and wind speed (Davis® Weather Monitor II™, Davis Instruments Inc.) on the recapture of marked *An. darlingi* females were analyzed using nonparametric bivariate analyses and multiple linear regression (SPSS version 9.0, SPSS Inc.). Samples of each species, by hour, from all collections were screened for malaria sporozoite infection using the VecTest™ rapid diagnostic kit according to manufacturers instructions (Medical Analysis Systems Inc., Camarillo, CA).

RESULTS

In order to ensure the experimental huts used in the present study were built using similar designs and materials of typical indigenous homes, a survey of 100 houses in the local (i.e., within 5 miles of the study site) village of Armenia was performed (Appendix II). The survey indicated homes had an average of 1.7 doors, 2.3 windows and a combined living/sleeping area of 15 ft. x 19.2 ft. Materials used for roofing included zinc (57%), thatch (36%) and zinc covered with oil shingles (7%). Walls were either made from wood planks (84%), cement blocks (7%) or a combination of the two (9%). The majority of floors were cement (48%) with 46% being dirt and 6% made from wood planks.

A total of 12 structures were constructed using the same materials as the experimental hut (i.e., zinc for roofs, wood planks for walls and dirt floors). Half of those structures were used only as sleeping quarters and had an average area of 14 ft. x 16 ft. This was similar to the experimental hut area of 13 ft. x 13 ft. Because we were interested in the biting behavior of *An. darlingi* throughout the night, we used an experimental hut area that was similar to that of the sleeping quarters of typical homes.

Three sampling trials (i.e., 6 all-night collections) were performed at each 0 M, 400 M and 800 M distance interval, for a total of 18 collections, during July 2002-June 2003. A total of 12,376 unmarked *An. darlingi* were collected during the all-night recapture collections (Table 1). Other anopheline species collected at the research site in decreasing densities included: 409 *An. albimanus* Wiedemann, 371 *An. pseudopunctipennis* Theobald, 28 *An. punctimacula* Dyar and Knab, 17 *An. vestitipennis* Dyar & Knab, and 1 *An. apicimacula* Dyar and Knab. In addition, 29 aberrant morphotypes of *An. darlingi* were also collected (Harbach et al. 1993).

Similar numbers of unmarked *An. darlingi* females were captured at the indoor stations (6,212) as the outdoor collection locations (6,164) giving an indoor to outdoor biting ratio of 1.00:0.99. Because of their importance in other behavioral studies in Belize, it should be noted that *An. albimanus* populations at the present study site were less endophagic than *An. darlingi* (i.e., indoor:outdoor ratio= 1.00:1.08), with 213 collected outdoors and 196 inside the experimental hut. On the contrary, *An. pseudopunctipennis* specimens were collected more often biting indoors (242) than at the outside station (129) giving an indoor to outdoor biting ratio of 1.00:0.53 (Table 1). No

positive sporozoite infections were detected in any of the individual species' pools from all 18 collections. This included the marked *An. darlingi* populations.

A total of 115 *An. darlingi* females were collected to serve as control populations, of which there was an overall 7.82% (9/115) mortality rate. All nine deaths occurred from one control population (9/20) of the first sampling trial at the 400 M site. A total of 459 *An. darlingi* specimens were dissected in parity rate examinations (Figure 5A-D). Of those 65% (299) were nulliparous, 29% (134) parous and 5.7% (26) were unidentifiable as a result of degradation. There were no significant differences in the number of nulliparous and parous females of the release populations within the sampling trials conducted at the 0 M (chi-square=0.213) or 800 M site (chi-square=0.087) (Figure 5A and C). The third trial at the 400 M site had significantly more parous females in the release population compared to the first two trials (chi-square=22.05; p=0.001) (Figure 5B). However, this difference had no effect on the recapture rate of *An. darlingi* (see below). Examination of the parity rates among distance intervals indicated the number of nulliparous and parous females released were similar (chi-square=1.44) (Figure 5D).

From nine resting collections, a total of 1,185 *An. darlingi* females were captured for mark and release (Table 2). A total of 428 were released from the 0 M site, 396 from the 400 M site and 361 for studies conducted at the 800 M distance interval. As expected, the total number of recaptured marked *An. darlingi* females decreased with increasing distance from the release point. Taking mortality into consideration, the total recapture rate at each distance included: 29.0% (124/428) at 0 M, 11.6% (37/318) at 400 M and 5.82% (21/361) at 800 M. Statistical analyses defined a significantly higher average number of recaptured females at the 0 M site compared to both the 400 M (ANOVA;

$p=0.060$) and 800 M (ANOVA; $p=0.022$) collection locations. No statistical difference was found between the 400 M and 800 M distances (ANOVA; $p=0.901$).

Examinations into the number of marked *An. darlingi* females recaptured at indoor versus outdoor stations indicated no significant differences between sampling trials at the same distance location (0 M; chi-square= 4.40 / 400 M; chi-square= 0.66 / 800 M; chi-square= 2.98) (Figure 6A-C). Because of this, the number of indoor and outdoor recaptures from all three trials at each distance were averaged and reanalyzed. No significant differences were seen when the average number of indoor and outside recaptures were compared among distance sites (chi-square=0.996) (Figure 6D).

Sampling trials were then separated into either Day 1 or Day 2 post-release at each distance site (Figure 7A-C). The total number of *An. darlingi* recaptured indoors versus outdoors was not significantly different within collection days. This was true for Day 1 collections at the 0 M (chi-square=5.11), 400 M (chi-square=0.200) and 800 M (chi-square=3.30) locations and Day 2 collections at the 400 M site (chi-square=1.89). However, the number of specimens recaptured inside (6) on Day 2 at the 0 M location was significantly higher than the three collected outdoors on the same day (chi-square=9.0; $p=0.025$). Analyses could not be performed for Day 2 data at the 800 M distance because of low sample size.

Data were then collapsed into either Day 1 or Day 2 indoor and outdoor collections and averaged for each distance. These averages were first compared within day of collection. When examined, analyses indicated no significant differences in Day 1 indoor/outdoor stations for 0 M (Student's t-test=0.516; $p=0.633$), 400 M (Student's t-test=-0.707; $p=0.519$) or 800 M (Student's t-test=0.381; $p=0.723$). Similar findings were

described for Day 2 collections at the 0 M (Student's t-test=0.480; p=0.656), 400 M (Student's t-test=0.378; p=0.725) and 800 M distance sites (Figure 6D). However, when Day 1 indoor and outdoor recaptures were compared to Day 2 collections within distances, significantly more marked *An. darlingi* were recaptured outside on Day 1 at the 0 M site (Student's t-test=5.277; p=0.006) than on Day 2. At the 400 M distance, both indoor (Student's t-test=3.536; p=0.024) and outdoor (Student's t-test=4.914; p=0.008) recaptures on Day 1 were higher than on Day 2. The numbers recaptured either indoors or outdoors at the 800 M site on Day 1 were not different than that of Day 2 (Student's t-test; t=2.335, p=0.064). Examination of Day 1 and Day 2 data among distances showed that the number of *An. darlingi* recaptured at the 0 M site on Day 1 outside was significantly higher (ANOVA; F=15.237, p=0.004) than those collected outdoors at either of the 400 M and 800 M distance (Figure 6D). No other differences were seen between inside collections on Day 1 or either indoor or outdoor collections on Day 2.

Although sometimes not significant (i.e., at the 800 M site), the majority of marked females recaptured during each sampling trial from all distances were collected on Day 1 post-release, while on the second night, consistently fewer were recaptured (Table 2). Using these data, further examination into the recapture pattern of marked *An. darlingi* individuals was undertaken. By dividing Day 1 data into three time periods including: early evening (i.e., hours 1-4); nocturnal (i.e., hours 5-9); and pre-dawn (i.e., hours 10-13), a variation could be seen in the time period within which the cumulative majority of marked *An. darlingi* had been recaptured (Table 3). At the 0 M and 400 M site the first time period was the most productive with 70% (81/115) and 83% (25/30) of the total night's recapture being collected, respectively. At the 800 M site the cumulative

majority was not reached until the second time period by which 68% (13/19) of the total night's recaptured were collected. Although the total numbers recaptured were low at each distance, these same trends were repeated for Day 2 collections where 100% (9/9) and 71% (5/7) were collected during the first time period at 0 M and 400 M, respectively (Table 3).

By graphing the total percent of recaptures made on Day 1 and Day 2 post-release by collection hour, more detailed patterns were revealed. The hourly recapture pattern of marked *An. darlingi* females at 0 M from the release point shows recaptures were made during all but two collection hours on Day 1 (Figure 8A). The majority (47%; 54/115) of the recaptures occurred at Hour 1 and there was another visible but weak peak at Hour 12 when 15% (17/115) marked females were collected. On Day 2 at 0 M, a similar pattern was seen in the early evening with 56% (5/9) of the recaptures occurring within the first collection hour (Figure 7A). However, no marked *An. darlingi* females were collected after four hours post-sunset and no morning peak was seen. Bivariate analyses indicate a significant correlation between the number of recaptured females and collection hour for both Day 1 ($r=-0.258$; $p=0.014$) and Day 2 ($r=-0.268$; $p=0.011$) sessions.

At the 400 M distance site, similar to that seen at the 0 M location, the majority (40%; 12/30) of marked specimens were collected in Hour 1 on the first day (Figure 8B). Two specimens were also recaptured during the pre-dawn hours on Day 1, although this did not represent a peak as seen at distance 0 M. On Day 2 at 400 M, 57% (4/7) of the marked females recaptured were collected at Hour 2, although one marked specimen was also sampled at Hour 12 (Figure 8B). The relationship between collection hour and total

recapture of marked females was significant on Day 1 ($r=-0.434$; $p=0.0001$) but not Day 2 ($r=0.155$; $p=0.093$).

Despite the low numbers of *An. darlingi* recaptured at distance 800 M, collection patterns could be described for Day 1 (Figure 8C). There was a distinct shift in the time of peak recapture compared to the 0 M and 400 M sites. The majority of recaptures were made in Hour 7, when 32% (6/19) were collected. In addition, marked females were captured from every collection period after five hours post-sunset, and two samples were taken at Hour 12. Because only two marked females were collected on Day 2 at 800 M, no patterns could be described (Figure 8C). However, it should be noted that one was captured at Hour 5 and another at Hour 12. No significant associations were found between hour of collection and total marked females recaptured for either Day 1 or Day 2 at the 800 M site. This is most likely an effect of the low number of recaptures made.

Examination of the biting patterns of unmarked *An. darlingi* populations sampled during recapture collections on Day 1 and Day 2 post-release indicate primarily bimodal activity with one predominate peak occurring within three hours post-sunset and a second, weaker peak sometimes occurring within the hour before sunrise (Figure 9 and 10). Total collections of unmarked females were highest at the 0 M distance site (6,501) compared to 2,903 and 2,970 at the 400 M and 800 M locations, respectively. This trend was true for both Day 1 and Day 2 collections, however, the total number of unmarked *An. darlingi* collected on Day 2 at 0 M and 800 M were less than those collected on Day 1. The 400 M site, however, sampled 877 on Day 1 and 2,026 Day 2.

On Day 1 specifically, populations at the 0 M and 400 M distances had peak biting occurring three hours post-sunset while the peak occurred one hour earlier at the

800 M site (Figure 9A-C). A much weaker peak occurred at all three distances during Hour 12 and interestingly, populations at 400 M and 800 M exhibited a substantial rise in biting activity at Hour 7. Overall, the unmarked populations exhibited an endophagic biting behavior with indoor to outdoor ratios on Day 1 ranging from 1.00:0.82 at the 400 M distance to 1.00:1.15 at the 0 M site.

Biting activity patterns for unmarked populations collected on Day 2 show peak biting occurring within three hours post-sunset for the 0 M and 400 M distances and within four hours post-sunset at the 800 M location (Figure 10A-C). Substantial biting peaks were also found in Hour 12 at 400 M and 800 M while no such peak was defined at 0 M from the release point. Overall, the unmarked populations exhibited an endophagic biting behavior with indoor to outdoor ratios on Day 2 ranging from 1.00:0.84 at the 800 M site to 1.00:0.96 at the 400 M distance location. There were no significant differences, as determined by the Kolmogorov-Smirnov two-sample test, between distances for both Day 1 and Day 2 natural biting activity patterns.

When the hourly recapture patterns of *An. darlingi* recaptured on Day 1 both inside and outside the hut are overlaid onto the all-night biting patterns of the “natural”, unmarked populations, several characteristics can be described (Figure 11A-C). First, the peak recapture period (i.e., Hour 1) at the 0 M distance occurs two hours prior to the peak in the biting of both the indoor and outdoor natural populations (Figure 11A). In addition, the smaller peak in indoor recapture at Hour 12 corresponds to an increase in the indoor biting population.

At the 400 M site, the peak recapture periods for both indoor and outdoor marked populations (i.e., Hour 1-2) on Day 1 occurred before the peak in the natural biting

activity (i.e., Hour 3) (Figure 11B). The weaker peak in the indoor biting of the unmarked population at Hour 12 corresponds to an indoor recapture, while when the other substantial biting peak of the natural population occurred at Hour 7, no recaptures of marked females were made.

Examination of Day 1 patterns at 800 M from the release site indicate a peak in the recapture of marked *An. darlingi* corresponding to an increase in both indoor and outdoor biting populations (Figure 11C). In addition, similar correspondence was seen during Hour 12 between indoor recaptures and natural biting activity. However, when the biting activity of the natural population was at it's highest during Hour 2, only one marked female was collected at each of the indoor and outdoor stations.

Although the total number of recaptures made during Day 2 collections was low at all distance locations, interesting results could be described when overlaid with the biting activity of the natural population (Figure 12A-C). At the 0 M distance site, overall recaptures were collected during the early evening hours. The peak in recapture occurred within the first collection hour, a full two hours earlier than the peak in the natural biting activity, and all were collected from inside the hut (Figure 12A). These patterns are similar to that of Day 1 collections (Figure 11A). However, unlike Day 1 no recaptures were made during the nocturnal hours when biting activity of the natural population remained elevated.

The biting pattern of recaptured females on Day 2 at the 400 M site indicated a peak at Hour 2 inside corresponding to a peak in indoor biting of the natural population (Figure 12B). In addition, a recapture made outside during Hour 12 corresponds to a weak peak in outdoor natural populations. However during collection hours 3-7, no

marked *An. darlingi* were recaptured even though biting remained elevated within the natural population.

Valuable information from Day 2 recapture collections at 800 M from the release site is difficult to obtain because only two marked specimens were recaptured. Despite this, it should be noted that one recapture made indoors at Hour 5 occurred during high natural populations inside the experimental hut (Figure 12C). Likewise, the recapture made outdoors at Hour 12 occurred when natural populations were elevated. More importantly, no recaptures were made in the early evening hours (i.e., Hour 1-3), when the first peak in natural biting activity occurs.

Analyses of environmental data were performed to determine the effect of indoor and outdoor temperature, indoor and outdoor humidity and wind speed on the recapture of marked *An. darlingi* females. Weather data for all 18 collections showed an average nightly temperature of 25°C (range=16.7-31°C) inside and 24°C (range=16-29°C) outside the hut during recapture hours. Average relative humidity indoors (89.7%) was lower than outside the hut (95.0%). Temperatures within the humidified chamber holding the control populations ranged from 14° C to 29° C, with an average of 19° C. Humidity ranged from 48% to 98%, with an average of 77%. Except for the second sampling trial conducted at the 0 M site during the movement of a cold front in April, prevailing winds came from the East to Southeast with an average high wind speed of 4.13 km/hr.

Examination of environmental variables during the release period (i.e., one hour prior to the start of the Day 1 recapture collection), indicate no significant differences in the average outside temperatures (ANOVA; $F=0.854$, $p=0.472$) or outdoor humidity levels (ANOVA; $F=0.854$, $p=0.472$) among the 0 M, 400 M and 800 M distance

locations (Figure 13A). The average high wind speed recorded at the 800 M site, however, was significantly greater than the wind speeds recorded at the 0 M (ANOVA; $F=34.7$, $p=0.001$) and 400 M locations ($p=0.006$) locations. In addition, the wind speed at 400 M from the release site was significantly higher than those at the 0 M distance ($p=0.038$) (Figure 13A). This is not surprising since the 0 M site was protected by the forest.

Overall, the average indoor (ANOVA; $F=0.352$, $p=0.710$) and outdoor (ANOVA; $F=0.268$, $p=0.994$) temperatures during recapture hours were not significantly different among the 0 M, 400 M and 800 M distance locations (Figure 13B). Similar results were seen in recorded indoor (ANOVA; $F=2.00$, $p=0.171$) and outdoor (ANOVA; $F=0.007$, $p=0.994$) humidity levels (Figure 13B). However, the nightly average high wind speed recorded at the 800 M site (6.54 km/hr) was significantly greater compared to that recorded at the 0 M distance (1.5 km/hr) (ANOVA; $F=7.646$, $p=0.004$). No differences in wind speeds were found between the 800 M and 400 M site as well as between 400 M and 0 M from the release site (Figure 13B).

Because the number of recaptured, marked *An. darlingi* varied between Day 1 and Day 2 collections post-release, detailed environmental analyses of collection days were also conducted. At the 0 M site, average indoor temperatures ranged from 22-26°C and outdoor temperatures ranged from 22-25°C. Humidity levels inside the experimental hut ranged from 89-96% and the outside levels ranged from 94-96%. The average high wind speed ranged from 0.4-1.9 km/hr. When compared between sampling trials within either Day 1 (Figure 14A) or Day 2 (Figure 14B) recapture collections, no significant differences in any of these parameters were found. However, when data from all three

trials were averaged for each day, the indoor humidity level (90%) on Day 1 was significantly lower than the average outdoor humidity level (95%) (Student' t-test=-10.874; p=0.002). No difference was seen within Day 2 averaged data. In addition, when each parameter was compared between Day 1 and Day 2 recapture sessions no significant differences were found (Figure 14C).

At the 400 M site, average indoor and outdoor temperatures ranged from 21-26°C. Humidity levels inside the experimental hut ranged from 89-94% and outside levels from 93-97%. The average high wind speed ranged from 3.5-6.0 km/hr. No significant differences occurred between the three sampling trials performed on Day 1 (Figure 15A). For Day 2 recapture collections, the weather station malfunctioned during the third sampling trial so no environmental data were recorded. However, the indoor and outdoor temperatures, humidity levels and wind speeds for the first and second sampling trials were not significantly different (Figure 15B). When data from the three trials for each recapture collection day were averaged, no significant differences were found between indoor and outdoor Day 1 weather parameters (Figure 15A). Within Day 2 data, only the average indoor humidity level (90%) was found to be significantly less than that measured outside (96%) the experimental hut (Student's t-test=-6.353; p=0.024). Overall comparisons between Day 1 and Day 2 averaged weather data revealed no significant differences (Figure 15C).

Weather data collected at the 800 M distance site indicated average indoor temperatures ranging from 19-27°C and outdoor temperatures ranging from 19-26°C. Inside humidity levels ranged from 89-91% and outdoor humidity levels from 90-97%. The average high wind speed recorded varied from 4-13 km/hr. When sampling trials

were compared for each recapture collection day, no differences were found in any of the environmental parameters for Day 1 trials (Figure 16A). However, on Day 2, the average inside and outside temperatures, both of 18°C, in the first sampling trial were significantly lower than the inside and outside temperatures, both of 26°C, in the second sampling trial (Figure 16B). When weather data from all three trials were averaged for Day 1 post-release, the indoor humidity level (87%) was significantly lower than the outdoor humidity level (95%) (Student's t-test=-4.70; p=0.009) (Figure 16C). No differences were seen in Day 2 averaged data for any of the environmental parameters. Likewise, no significant differences were found when Day 1 temperature, humidity and wind speed averages were compared to Day 2 averages (Figure 16C).

In order to determine how temperature, humidity and wind speed may affect the time of recapture of marked *An. darlingi* females, hourly recapture patterns were overlaid onto hourly environmental patterns for both Day 1 and Day 2 post-release collections. On Day 1, the total number of indoor and outdoor recaptured females at the 0 M site was well correlated with the average hourly temperature ($r=0.270$; $p=0.011$) (Figure 17A). A significant relationship between temperature and hourly recapture rates was also seen at the 400 M distance ($r=0.366$; $p=0.0001$) (Figure 17B). However, hourly temperatures had no significant relationship with recaptures made at the 800 M site ($r=0.063$; $p=0.300$) (Figure 17C). This is most likely due to the low number (19) of total recaptures made at this site for Day 1.

The average hourly humidity levels on Day 1 were significantly associated with total recaptures made at the 0 M distance site ($r=-0.391$; $p=0.001$) (Figure 18A). This same negative relationship was found at 400 M from the release site ($r=-0.523$; $p=0.001$)

(Figure 18B), but the hourly recapture rate at the 800 M location was not significantly associated with average humidity levels ($r=0.048$; $p=0.344$) (Figure 18C).

Examination of the relationship between hourly average high wind speed and recapture rates on Day 1 indicate a strong effect of wind during collections made at the 0 M distance ($r=0.368$; $p=0.001$) (Figure 19A). The number of hourly marked *An. darlingi* females recaptured at the 400 M site also had a significant association with hourly wind speed ($r=0.235$; $p=0.024$) (Figure 19B), but no such relationships were seen at 800 M from the release site ($r=0.018$; $p=0.441$) (Figure 19C).

On Day 2 collections, no associations were found between average temperatures and total recaptures made at the 0 M ($r=0.190$; $p=0.06$), 400 M ($r=0.122$; $p=0.200$) or 800 M ($r=-0.085$; $p=0.239$) distance locations (Figure 20A-C). Examination of relative humidity levels indicated a significant relationship with only those recaptures made at the 400 M site ($r=-0.264$; $p=0.032$) (Figure 21B). No significant effects were seen at either the 0 M ($r=-0.067$; $p=0.307$) or 800 M ($r=0.054$; $p=0.326$) distance locations (Figure 21A,C). In addition, associations between hourly wind speed and recapture of marked specimens at all three distances were insignificant (0 M; $r=-0.015$, $p=0.450$ / 400 M; $r=0.033$, $p=0.412$ / 800 M; $r=0.120$, $p=0.157$) (Figure 22A-C). Once again, this is most likely an effect of low sample sizes.

Because there existed a variation in the peak recapture time on Day 1 collections (i.e., Hour 1 at 0 M; Hour 1-2 400 M; Hour 7 at 800 M (Figure 8A-C)), differences in the environmental data for these specific hours were examined in order to determine if the variation could be described by average temperature, humidity and/or wind speed. Recapture rates on Day 2 collections at all distances were not defined by substantial time

trends, due to low sample sizes, therefore no statistical analyses of hourly environmental data were performed.

On Day 1 collections, the average indoor temperatures at Hour 1 (ANOVA; $F=0.136$, $p=0.876$), Hour 2 (ANOVA; $F=0.113$, $p=0.895$) and Hour 7 (ANOVA; $F=0.928$, $p=0.445$) were not significantly different among distances. Similar results were seen among average outdoor temperatures at Hour 1 (ANOVA; $F=0.165$, $p=0.852$), Hour 2 (ANOVA; $F=0.220$, $p=0.809$) and Hour 7 (ANOVA; $F=0.556$, $p=0.600$). Comparisons among average indoor humidity levels on Day 1 also found no significant differences at Hour 1 (ANOVA; $F=1.234$, $p=0.356$), Hour 2 (ANOVA; $F=2.681$, $p=0.147$) or Hour 7 (ANOVA; $F=2.833$, $p=0.136$). The mean differences in outdoor humidity levels were also insignificant at Hour 1 (ANOVA; $F=0.327$, $p=0.735$), Hour 2 (ANOVA; $F=0.172$, $p=0.847$) and Hour 7 (ANOVA; $F=0.853$, $p=0.480$).

When the average outdoor high wind speeds of the specific collection periods were analyzed, a significantly higher measurement was indicated for Hour 1 at the 800 M site (14.0 km/hr) compared to the 0 M site (3.7 km/hr) (ANOVA; $F=10.296$, $p=0.010$). This was also true for the average 8.6 km/hr wind speed recorded at 800 M during Hour 2 compared to the 2.1 km/hr recorded at 0 M (ANOVA; $F=7.077$, $p=0.022$). However, no significant differences were described between wind speeds at the 800 M and 400 M locations for either Hour 1 ($p=0.067$) or Hour 2 ($p=0.054$). In addition, no differences were seen in Hour 7 data among the 0 M (0.53 km/hr), 400 M (4.8 km/hr) or 800 M (2.67 km/hr) locations (ANOVA; $F=2.824$, $p=0.137$).

DISCUSSION

The transmission of malaria depends on the contact between human host and anopheline vector. Factors influencing the intensity of this contact include: vector habitat availability; population densities; biting patterns; and flight behavior. Therefore, an understanding of the natural history behind these factors is vital in reducing malaria in endemic areas. Results of the present research, which focused on describing the change in *An. darlingi* recapture densities at various distances from a fixed release point, is the first to report on the flight behavior of this important vector in Belize. In addition, the systematic design incorporating a portable experimental hut is a novel approach.

Anopheles darlingi is one of four anopheline species incriminated in the transmission of malaria in Belize. This is based upon natural sporozoite infections (Kumm and Ram 1941; Achee et al. 2000), an endophagic feeding behavior (Grieco 2001; Roberts et al. 2002), all-night biting patterns (see Chapter 2) and its role in malaria transmission throughout its geographic distribution (Deane et al. 1948; Foot and Cook, 1959; Arruda et al. 1996). Although generally accepted as a primary malaria vector, surprisingly only a handful of studies, all from South America, have described the flight behavior of this important species (Deane et al. 1948; Charlwood and Alecrim 1989). There has been no past research into the flight behavior of *An. darlingi* in Belize.

Without previous data from Belize to review, it was not known to what level *An. darlingi* would be recaptured. Mark-recapture studies of other anophelines have reported rates ranging from 0-38% (Service 1993). Of course this will depend on the individual species and study design. The overall recapture rate of 16.4% (182/1,107) reported in the present study is high compared to the recapture rates of several other anopheline species

(Service 1993). Even though the individual recapture rates of 29.0% at the 0 M, 11.6% at the 400 M and 5.8% at the 800 M locations decreased with increasing distance as expected, values at all sites clearly indicate the anthropophilic nature of *An. darlingi*. These results are similar to the 12% recapture rate for *An. darlingi* collected 0-70 M from a house at a study site in Brazil (Charlwood and Alecrim 1989). The average recapture rate of 2.3% for nine other species from the Charlwood and Alecrim study was much smaller.

By taking into consideration the total area within the established buffer zones at the present study site, an even more pronounced attraction of *An. darlingi* to humans is apparent. The area within the 400 M buffer zone totals 0.50 km² and that of the 800 M buffer zone totals 2.00 km². This represents an increase in area of approximately 0.50 km² and 1.50 km² from the 0-400 M and 400-800 M distances, respectively. The change in area between the 0 M and 800 M sites is a four fold difference, while the number of marked specimens recaptured at each site was only decreased by half of that collected from the preceding distance starting from the 0 M site.

The anthropophilic behavior exhibited by recaptured specimens was not a result of the absence of a bloodmeal host. Although no other human hosts inhabited the land within each distance buffer zone, other wildlife (i.e., tapir, monkey, porcupine, gibnut, armadillos, koatamundi, etc.) abounded in this area and particularly within the forest swath from which the marked specimens were released. Even when other bloodmeal sources were available, marked *An. darlingi* continued to seek human hosts as far as 800 M (i.e., a search area of 2.0 km²) from the release site. Rozendaal (1987) similarly reported the tendency of *An. darlingi* females to seek hosts inside human settlements

rather than in forests. Such human host-preference has been documented from behavioral studies in Brazil where 65% (421/649) of *An. darlingi* were captured from human hosts versus adjacent animal bait (Oliveira-Ferreira et al. 1992). Similar reports from the southern Toledo District of Belize have identified the bloodmeal source from all resting, engorged *An. darlingi* specimens as human, although the sample size was small (Grieco 2001). However, in the present study it must be stated that the majority of released specimens were not recaptured and dispersed throughout the area. In order to quantify the level of *An. darlingi* human host-preference, detailed, systematic bloodmeal analyses are needed.

The overall decrease in the recapture rate of *An. darlingi* with increasing distance is not suggested to be a factor attributed to the marking process. The same marking technique was used for all release populations and if the method interrupted the flight behavior it would be expected that the recapture rate at the 0 M site would not have been so high. In addition, the mortality rates within the control populations were minimal, with a total of nine mosquitoes dying throughout the entire study, all of which were from one population held for Trial 1 at the 400 M distance. These deaths were apparently caused by bacterial growth on the sugar pads.

Similarly, differences in the recapture rates among distances reported in the present study cannot be attributed to the physiological stage of the release populations. First, resting females were not marked if they contained a bloodmeal or partial bloodmeal. Next, as defined by the gonotrophic state, the resting *An. darlingi* populations used for marking were similar in age (i.e., nulliparous). The youngness of the females used in the study is most likely a result of the study design. Trials were only conducted

when there were sufficient numbers of resting *An. darlingi* to validate a recapture study. This required a resting collection of at least 105 females (i.e., release population=50, control population=5 and parity rate population=50). Such numbers were not consistently available at the study site, reflected in the eight month time period required to complete the study. When the required resting population was available it was most likely a result of a recent emergence of adults. However, in Brazil, Charlwood and Wilkes (1979) found that the majority (64.4%; 486/755) of *An. darlingi* caught at human bait during early evening hours were nulliparous. While this represents a biting population, similar patterns may be seen in the resting population. If true, this may explain why more nulliparous females were collected for marking treatment than parous adults. Most importantly, results indicate the one release population that contained a higher rate of parous females (i.e., Trial 3 at 400 M) were recaptured in similar numbers (14) as that for Trial 1 at the same distance and more than the nine collected in Trial 2.

However, this does not mean that the physiological stage would not influence the flight behavior of a vector. Studies of *An. darlingi* in Brazil have shown that when females were allowed to feed before release, the overall recapture rate of the engorged population (14%) was lower than the rate (19%) for a population that had been released unfed (Charlwood and Alecrim 1989). In addition, marked females that had been blood fed 24-hrs prior to release were recaptured at higher rates close to the release site versus the other collection sites ranging from 30-70 M away. When performed over several days, such data can provide insight into both the survival rates and gonotrophic cycles (i.e., bloodmeal-oviposition interval) of a vector species. A higher survival rate and shorter gonotrophic cycle can result in increased frequency of vector-host contact and

increased risk of disease transmission. For this reason it would be extremely interesting for future efforts in Belize to examine differences in the recapture rates of *An. darlingi* populations under various physiological stages at several distances from a release site.

Overall, there was no difference in the number of marked *An. darlingi* females collected inside versus outside the experimental hut at any of the distances. This was seen even though outdoor collectors were positioned at a parallel with the edge of the house facing the release site in order to ensure equidistance to indoor and outdoor host sources. The results are not surprising considering the indoor to outdoor biting ratio of the natural unmarked population at the study site was 1.00:0.99, or almost equal. The biting ratio clearly indicates that *An. darlingi* will enter homes to feed. This behavior has previously been documented for this species in Belize (Grieco 2001; Roberts et al. 2002; also see Chapter 2). However, these results provide insight into the resting and host-seeking behavior of *An. darlingi* prior to a bloodmeal.

If *An. darlingi* rested outdoors prior to entering a house, then it would be advantageous for that female to feed on a host source located outside if available. This would result in higher outdoor than indoor recapture rates which did not occur during the present research. Even at the furthest 800 M distance when the marked females would have had to expend energy to reach the site, recaptures indoors and outdoors were similar. Likewise, if females were attracted to the house structure alone (i.e., odor concentration or shadow effect) and then entered to feed, an increase in indoor recaptures would have resulted. This suggests that *An. darlingi* females will enter homes to rest prior to feeding, but the probability is equal to outdoor resting and feeding. Studies in Suriname have reported *An. darlingi* resting inside houses at night for only 7.7 min prior

to biting (Hudson 1984). No females were found resting by day, in buildings or out of doors. Charlwood (1980) reported that *An. darlingi* mosquitoes from Brazil rest within the vicinity of the host for up to 10 minutes before biting. No studies up to the present have focused on the adult resting behavior of *An. darlingi* in Belize. This information is vital for control operations based on a house-spray program. Future efforts should focus on the time and location of *An. darlingi* resting populations before and after a bloodmeal.

The total number of *An. darlingi* recaptured on Day 1 post-release was higher than on Day 2 at each of the 0 M, 400 M and 800 M sites. In addition, the number recaptured on Day 2 decreased with increasing distance. Similar results were reported from Brazil when 30% of the total recapture of marked *An. darlingi* females was collected on the first day following release and at the closest collection distance to the release site (Charlwood and Alecrim 1989). For the present study, it is suggested that those marked females that did not search out a collector for a bloodmeal, and subsequent recapture, on Day 1 had experienced natural predation, had taken blood from another source or had acquired a sugar meal post release and were not in host-seeking mode. This would result in an overall reduction in the Day 2 marked host-seeking population and decrease recaptures. Although the number of marked females collected on Day 2 was less than on Day 1, it is important to note that recaptures were still made on the second night after release. Considering the total surface area incorporated into the 400 M and 800 M buffer zones, these results provide additional evidence of the anthropophilic nature of *An. darlingi*.

In addition to overall recapture rates, differences were seen in the peak time of recapture between distances, most likely reflecting the increased time required to reach

the corresponding 400 M and 800 M sites. These variations were not associated with differences between hourly temperature or humidity levels, but the average wind speed was significantly different between the 0 M and 800 M sites for Hour 1 and Hour 2. The hut was strategically placed along a consistently NNE linear transect from the release site, so the NNE direction of the prevailing winds was blowing away from the release site and towards the hut at all distance sites; however, the tree line adjacent to the 0 M site most likely had a protective effect from winds. Still, the wind speeds between 0-400 M and 400-800 M located in open pastureland were similar for these time periods and recapture rates still differed. In addition, it should be noted that while the strongest winds were recorded during the first collection hour at all distance sites, the time period from which the majority of all recaptures were made (i.e., three hours post-sunset) at the 0 M and 400 M sites had the highest average wind speed of 5.9 km/hr. More importantly, the wind speed at Hour 7, during the peak time in recapture at the 800 M site, was not different than those recorded at the other distance locations. A caveat to these conclusions is necessary because wind speed was measured at the roof of the hut and most likely does not represent ground level speeds that may be more important in mosquito flight patterns. Future work should record wind speed at lower heights.

The peak time in which marked specimens were recaptured on both Day 1 and Day 2 varied from the biting pattern of the natural, unmarked *An. darlingi* population at each distance. Such differences are likely due to the location of resting sites of females prior to host seeking. Because the biting patterns of the natural population was similar at the 0 M, 400 M and 800 M sites for both Day 1 and Day 2 collections, the distance these females traveled from resting sites to each of the hut locations was similar. If the marked

populations rested at a different location than the natural females, then the time of peak recapture would vary because the distance to the hut would differ.

Marked females most likely rested within the forest swath from which released due to favorable diurnal humidity and temperature levels. Although the release site was purposely positioned at a location adjacent to the Sibun River with known *An. darlingi* larval habitats, this does not mean that other breeding habitats were not available at other places surrounding the study site. A general survey was conducted prior to the study, and most habitats were located sporadically within the adjacent Sibun River. These habitats are transitory, and the positions will fluctuate with the river based upon environmental events (see Chapter 2). Other sources of *An. darlingi* breeding sites have been reported from Belize including lagoons, small lakes and ground pools with floating vegetation (Manguin et al. 1996). Reports from South America have also found small lagoons to contain *An. darlingi* larvae (Rejmankova et al. 1999). While dependent upon rainfall, these habitats are not subject to positional changes as those within the river, therefore, the distance from these habitats to particular sites would remain unchanged.

Upon further examination of the study site using a 2002 IKONOS (4-m multispectral resolution) satellite image, a small lagoon was found equidistant at approximately 400 M from each of the distance locations. If some natural populations were from this area, the biting patterns would not differ between distances. Sampling of this lagoon was not successful in retrieving *An. darlingi* larvae, but a more thorough survey may have revealed presence of larvae (De Bustamante et al. 1948).

Another consideration in the variation between peak recapture and natural biting patterns could be attributed to a pre-positioning behavior of the natural host-seeking

population towards a house caused by a combination of environmental and host cues.

This is not hard to imagine when considering the sporadic nature of detritus mat formation (i.e., preferred breeding sites) within a river system (refer to Chapter 2, 4 and 6). Such patchiness in distributions of larval habitats will lead to large variation in distances to human hosts, resulting in requirements for adults to move towards human habitations prior to the initiation of feeding. In order to determine this behavior, a systematic quantification of *An. darlingi* resting population densities at fixed sites within various distances from an experimental hut would have to be performed over time. This would provide insight into positional time trends. Until then, it is not known if females become pre-positioned around homes through a series of small distance flights, or if this is accomplished using a few long distance flights. Likewise the timing of pre-positioning flight initiation is unknown. It is clear from collection data arrayed over distance and time that there are differences between recaptures of marked specimens and the times of peak biting of natural populations. Two hypotheses for these differences are that the discrepancies are artifacts of the mark and release conditions, or that differences simply reflect normal time and space factors that routinely account for natural patterns of biting activity.

Biting and recapture behavior of *An. darlingi* can be generalized to indigenous homes within Belize because the same design and materials were used to construct the experimental huts as those used for local housing. However, insecticides on house walls will influence vector behavior (Rozendaal et al. 1989; Grieco et al. 2000), and future studies should examine changes in recapture rates of *An. darlingi* as it relates to insecticide application. This would prove useful in evaluating present control operations.

Information from the present research should be applicable for malaria risk-assessment at the village level based on a combination of *An. darlingi* adult densities and distances to potential breeding sites. Clarifying the level of risk of disease will guide better decision-making processes needed for management and control by refining resource allocations.

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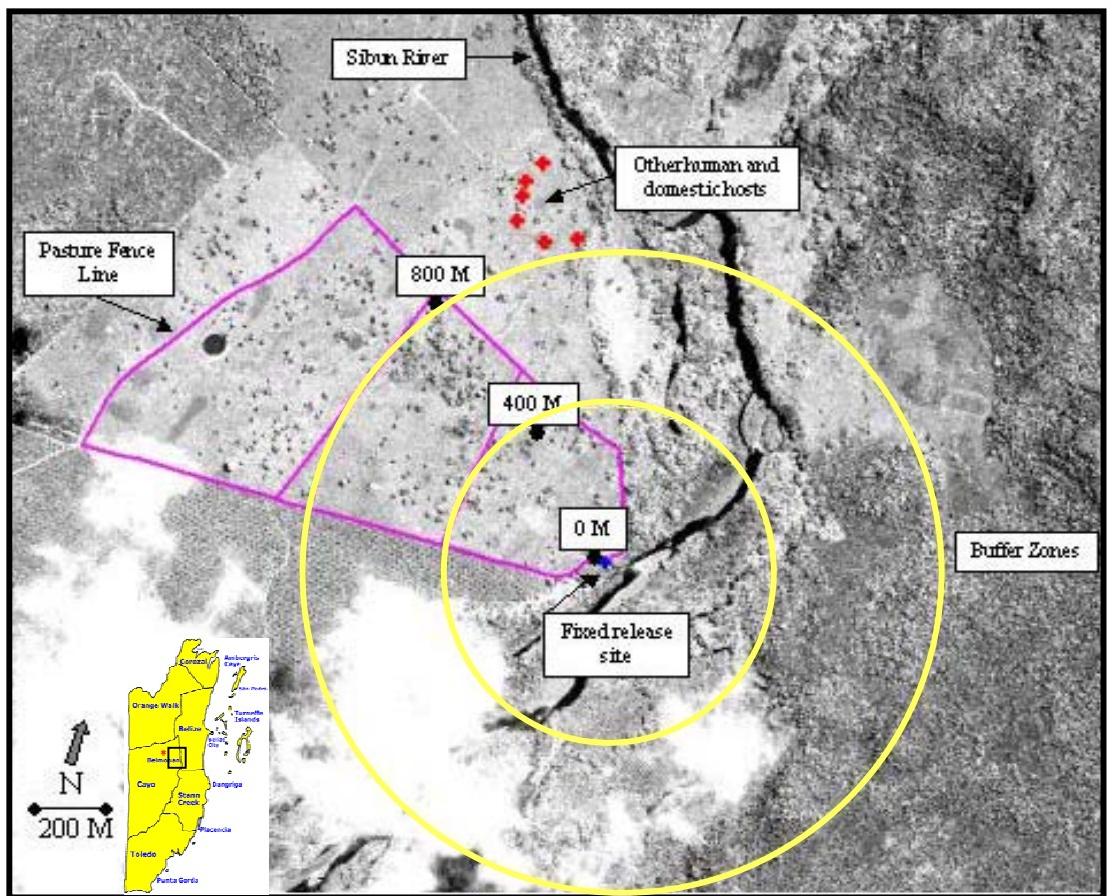


Figure 1. Study site in the Cayo District of Belize where a mark-release study of *An. darlingi* was conducted. An experimental hut was placed at either 0 M, 400 M or 800 M from fixed release site. Other human and domestic hosts were outside of the corresponding buffer zones. Surrounding landscape included the Sibun River, secondary forest, orchard and pastureland. Overlay was placed onto the 1-m panchromatic band of an IKONOS satellite image acquired in May 2002.

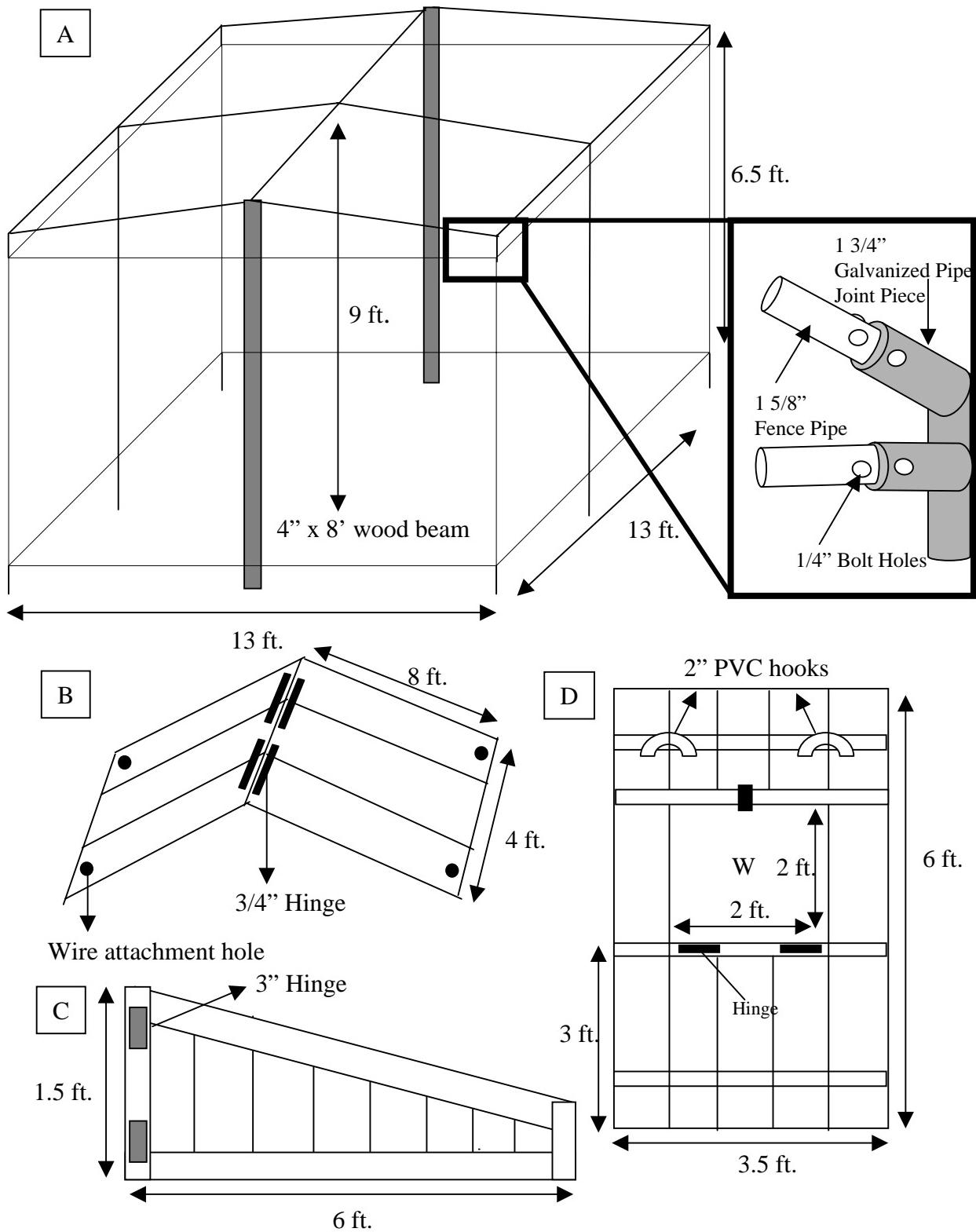


Figure 2. Portable experimental hut design. (A) Pipe infrastructure with inset detailing points of attachment between fence pipe and permanent joint pieces. (B) Individual hinged roof panel. (C) Individual gable panel (Inside view). (D) Individual wall board panel with hinged window (W).



Figure 3. Portable experimental hut used in a mark-release-recapture study to define the flight behavior of *An. darlingi*.

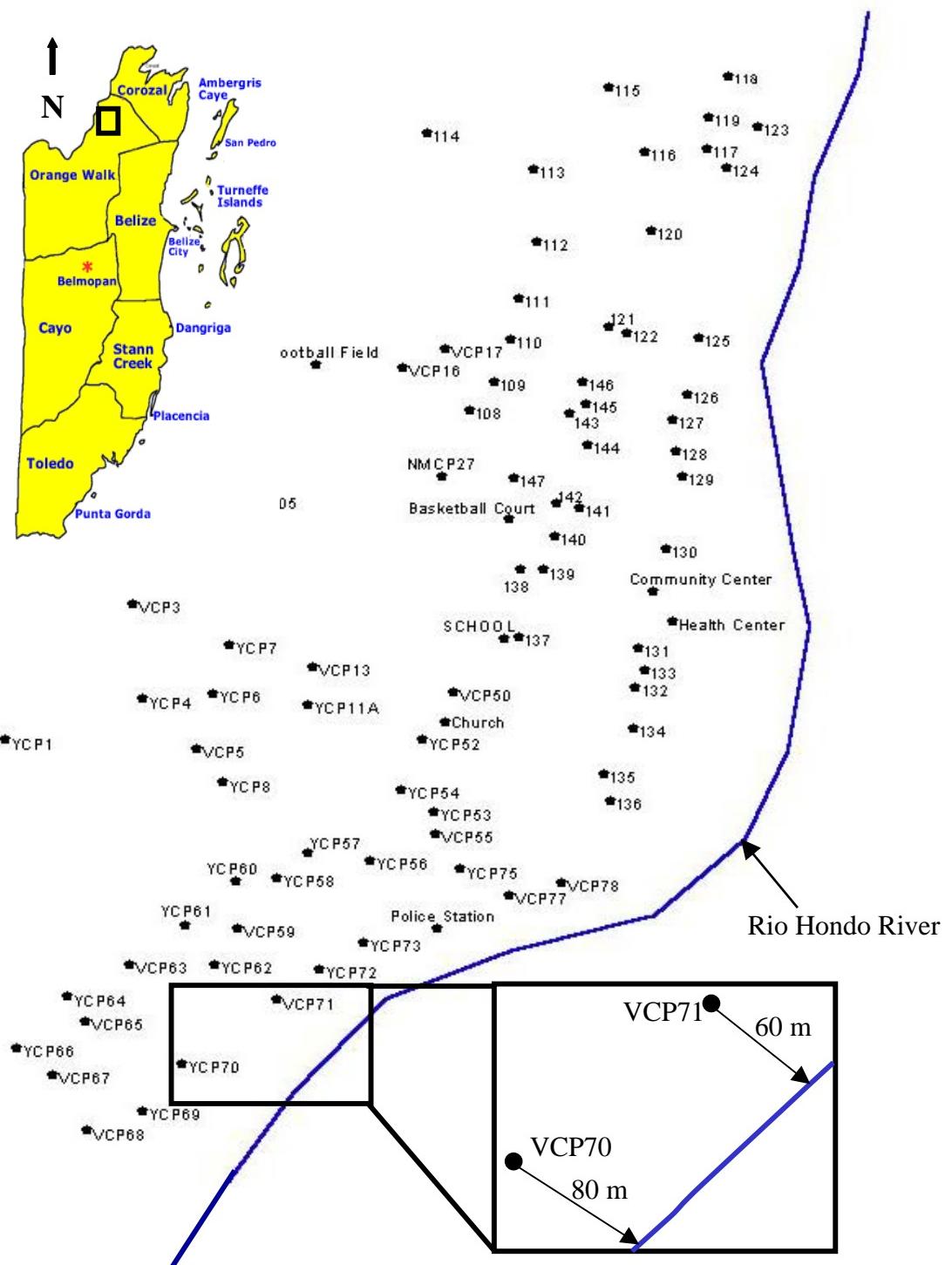


Figure 4. Graphic illustration of San Roman village in northern Orange Walk District of Belize (map inset). Structures within the village and a section of the Rio Hondo River were mapped using hand-held GPS units. The shortest, straight-line distance from each house to the river (village inset) was then calculated using GIS software and distance quartiles established for use in flight distance studies. VCP=Vector Control Program house number.

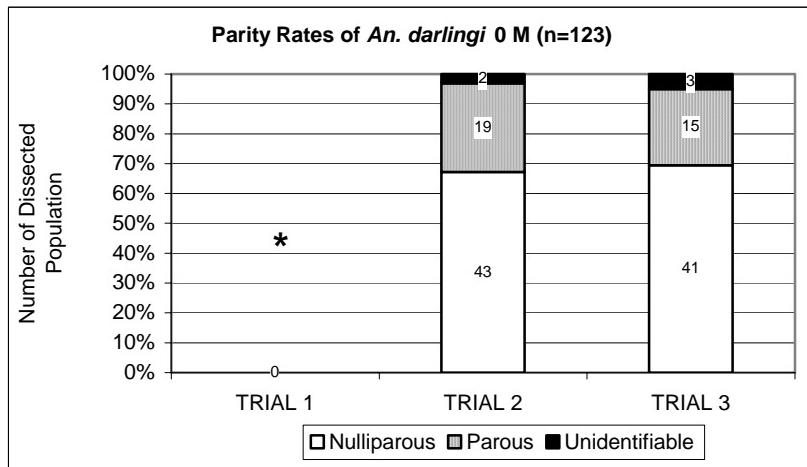
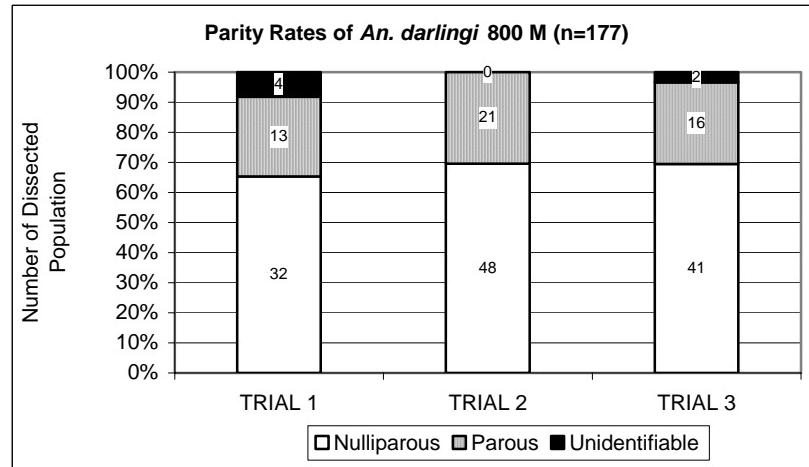
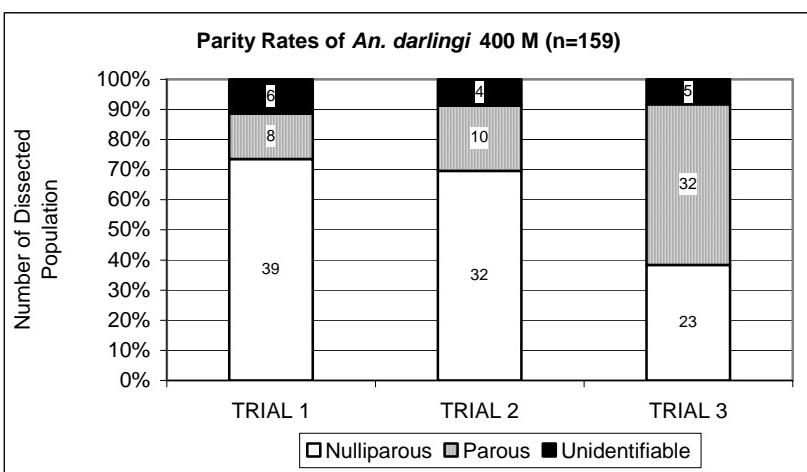
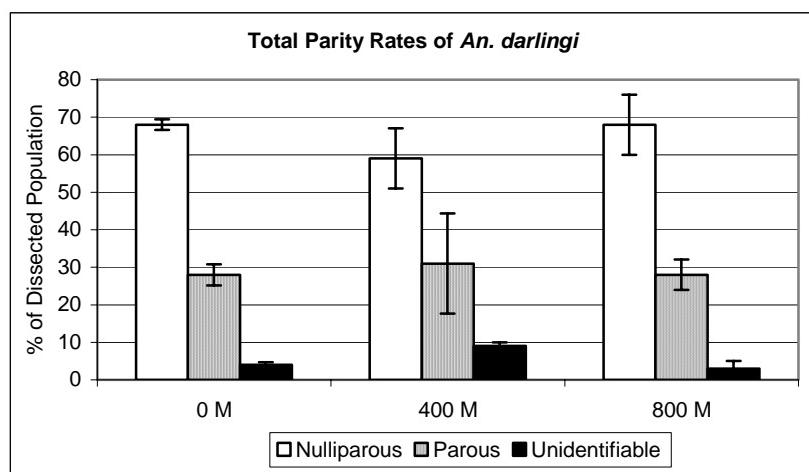
A**C****B****D**

Figure 5. The distributions of nulliparous and parous *An. darlingi* females released at either 0 M, 400 M or 800 M from a fixed release point for each sampling trial (A-C). *Slides from Trial 1 at 0 M were destroyed by ants and therefore not available for examination.(D) Represents the total percent distribution of all three trials at each distance.

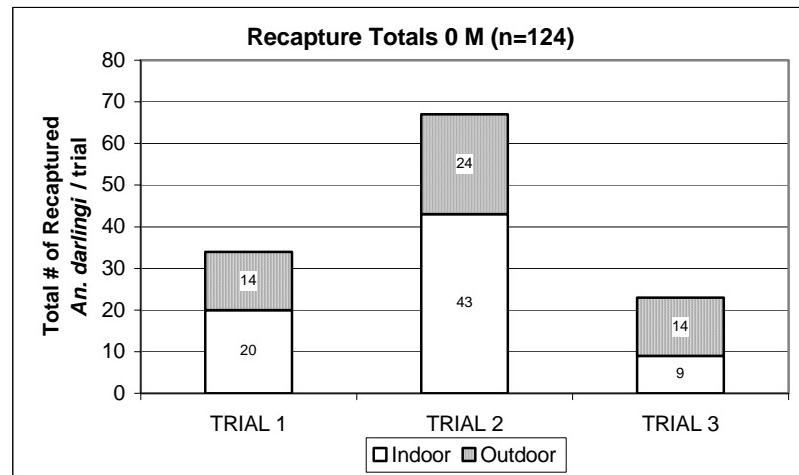
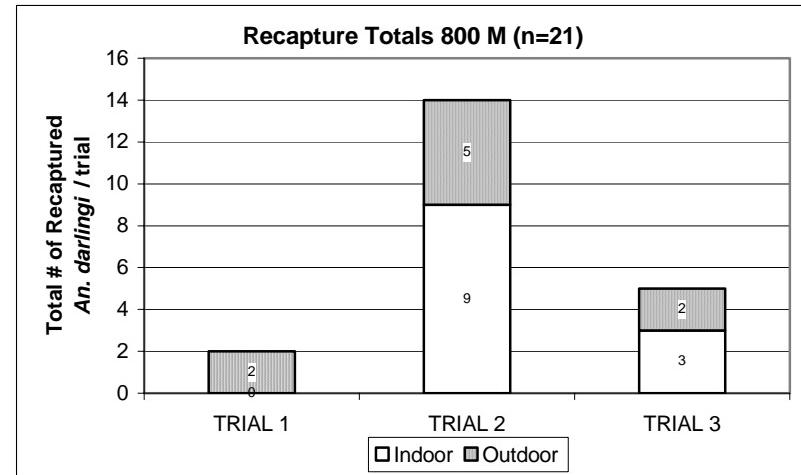
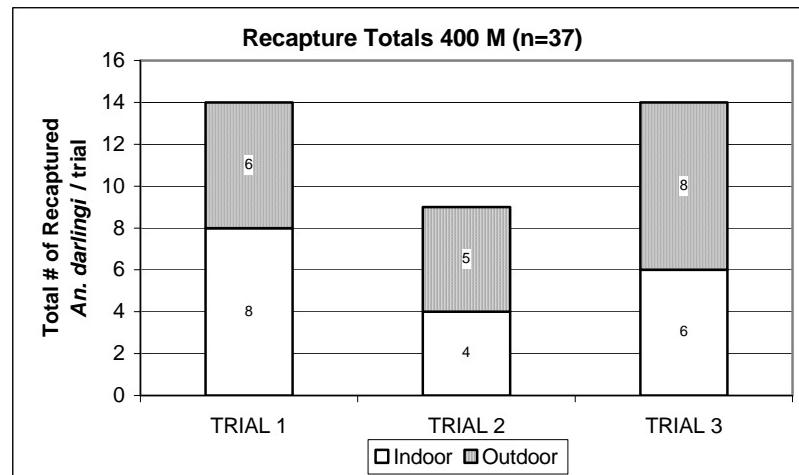
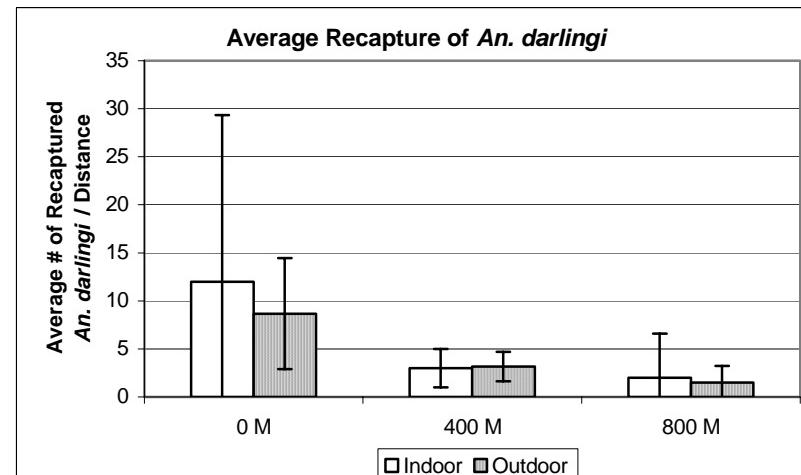
A**C****B****D**

Figure 6. The individual distributions of indoor and outdoor recaptured *An. darlingi* females for each sampling trial collected at either 0 M (A), 400 M (B) or 800 M (C) from a fixed release point. (D) Represents the total average distribution of marked specimens for each distance by collection station. Each trial consisted of two all-night collections.

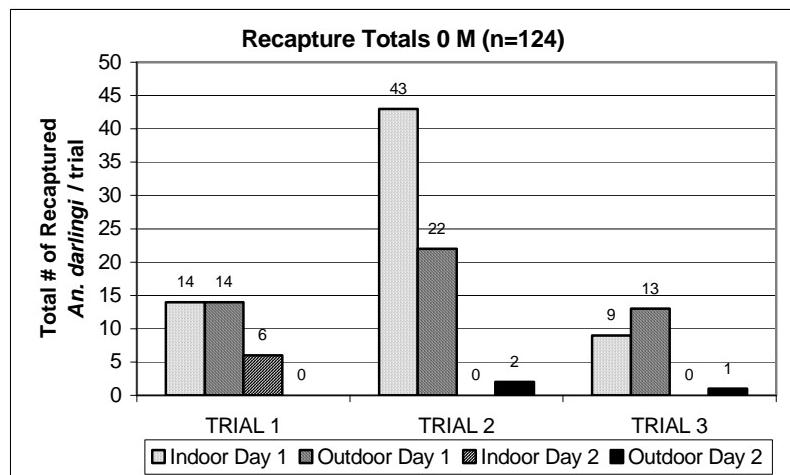
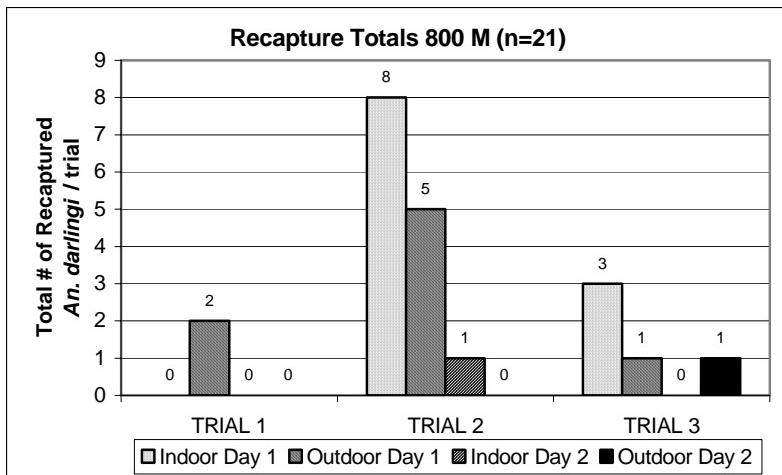
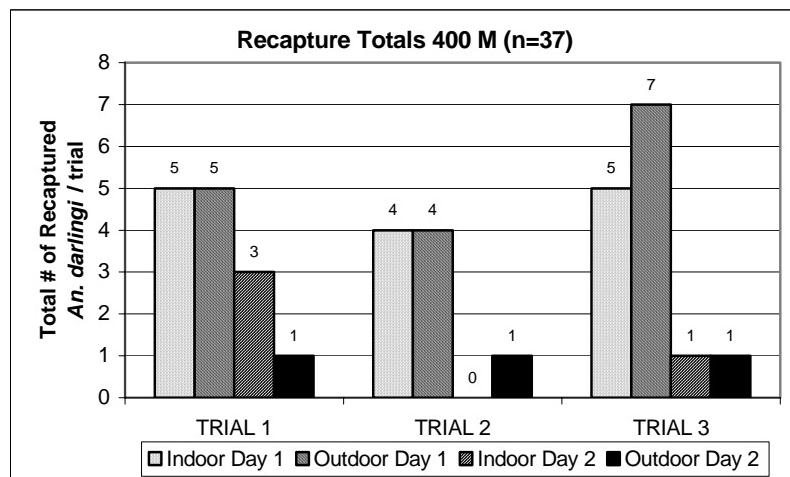
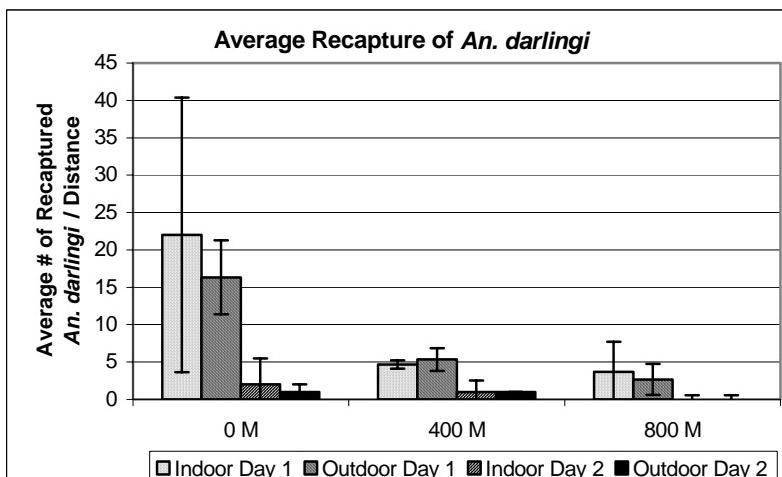
A**C****B****D**

Figure 7. The individual distributions of indoor and outdoor recaptured *An. darlingi* females for each collection day of each sampling trial conducted at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point. (D) Represents the total average distribution for all three trials at each distance. Each trial consisted of two all-night collections.

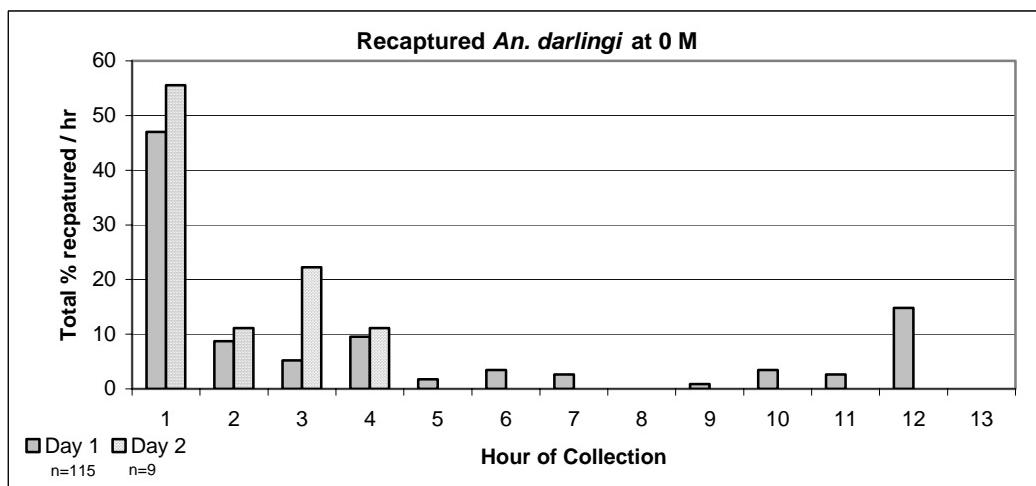
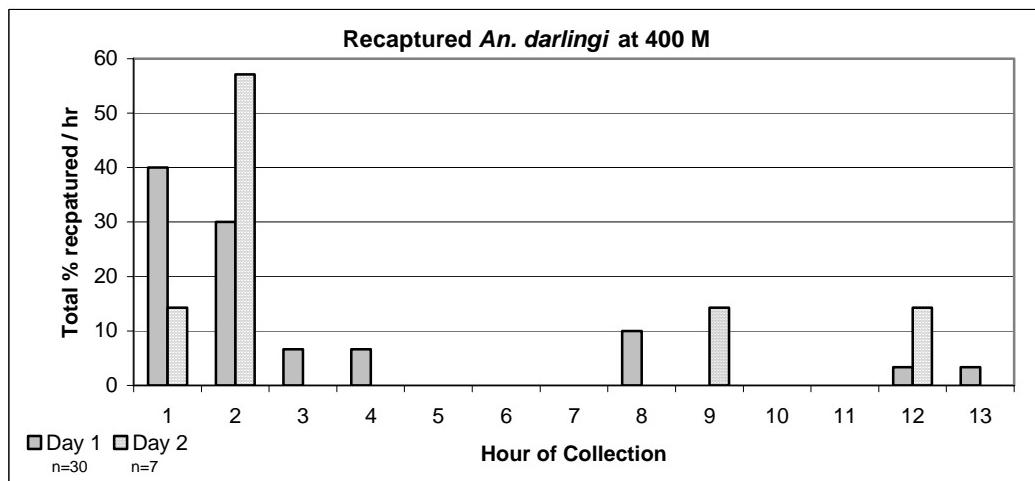
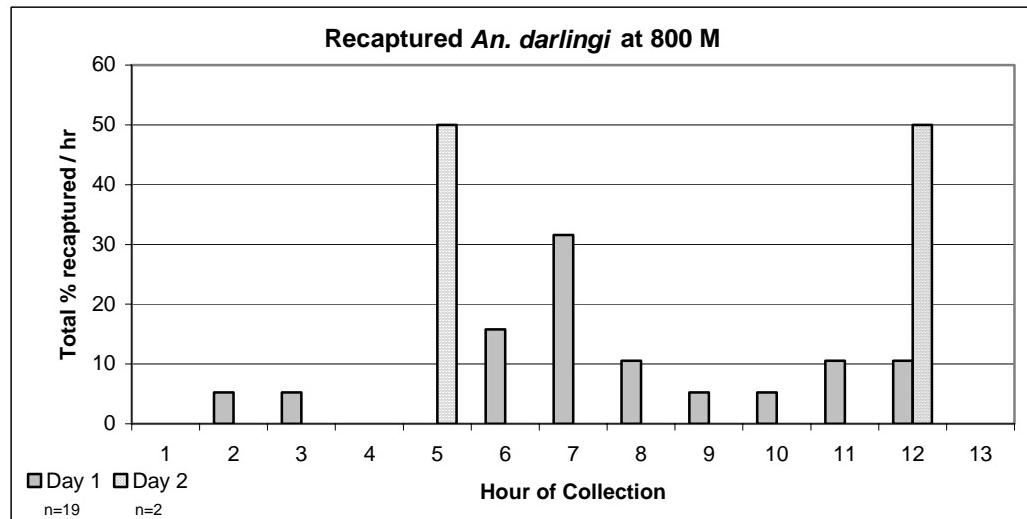
A**B****C**

Figure 8. Total percent of combined indoor and outdoor marked *An. darlingi* recaptured by collection hour and day at either (A) 0 M; (B) 400 M; or (C) 800 M from a fixed release site.

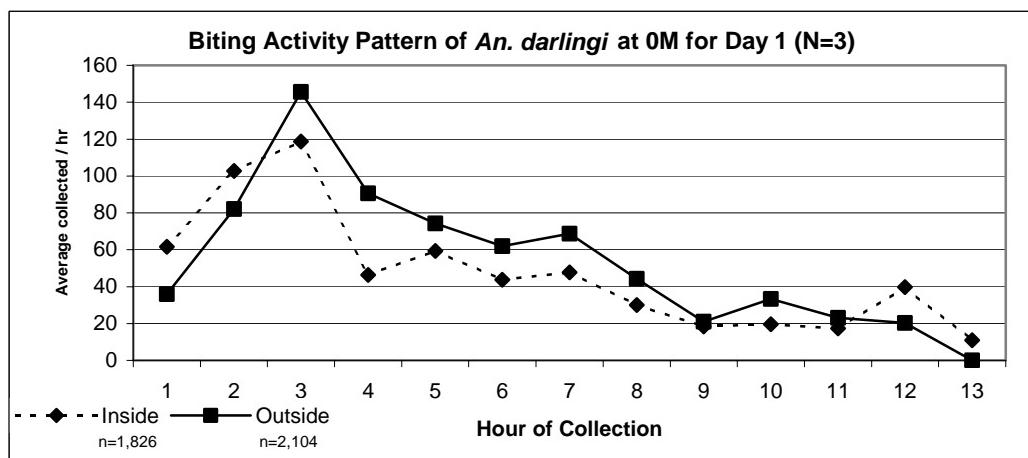
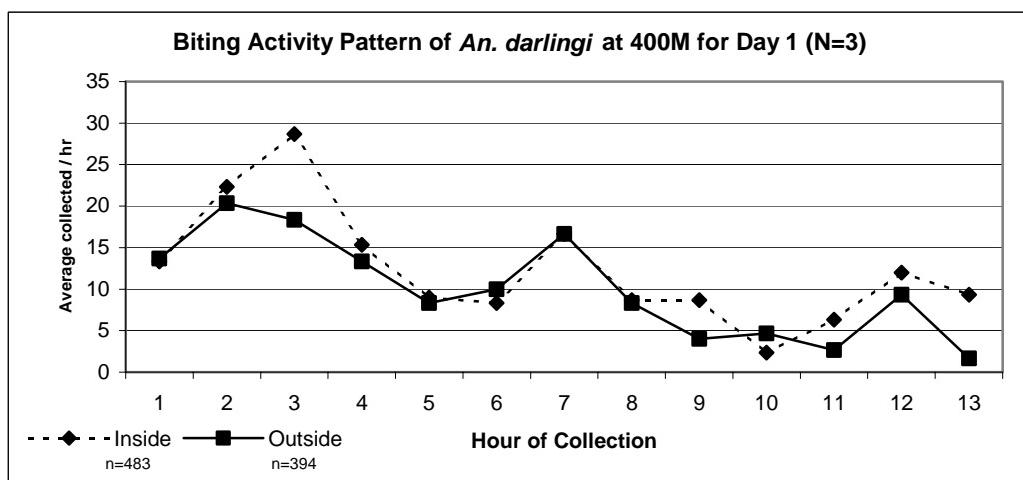
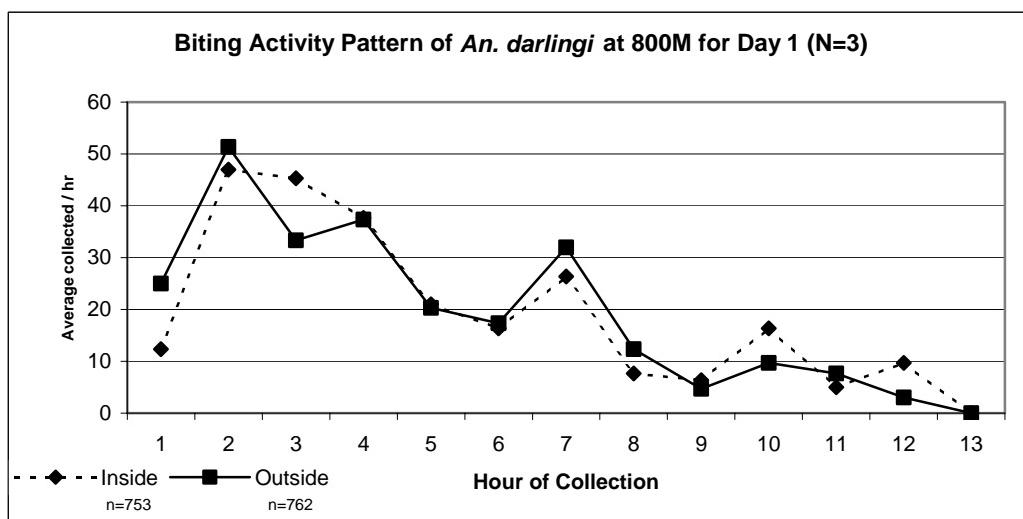
A**B****C**

Figure 9. Biting activity patterns of unmarked *An. darlingi* females captured on Day 1 post-release at either (A) 0 M, (B) 400 M, or (C) 800 M from a fixed release site during July 2002-May 2003.

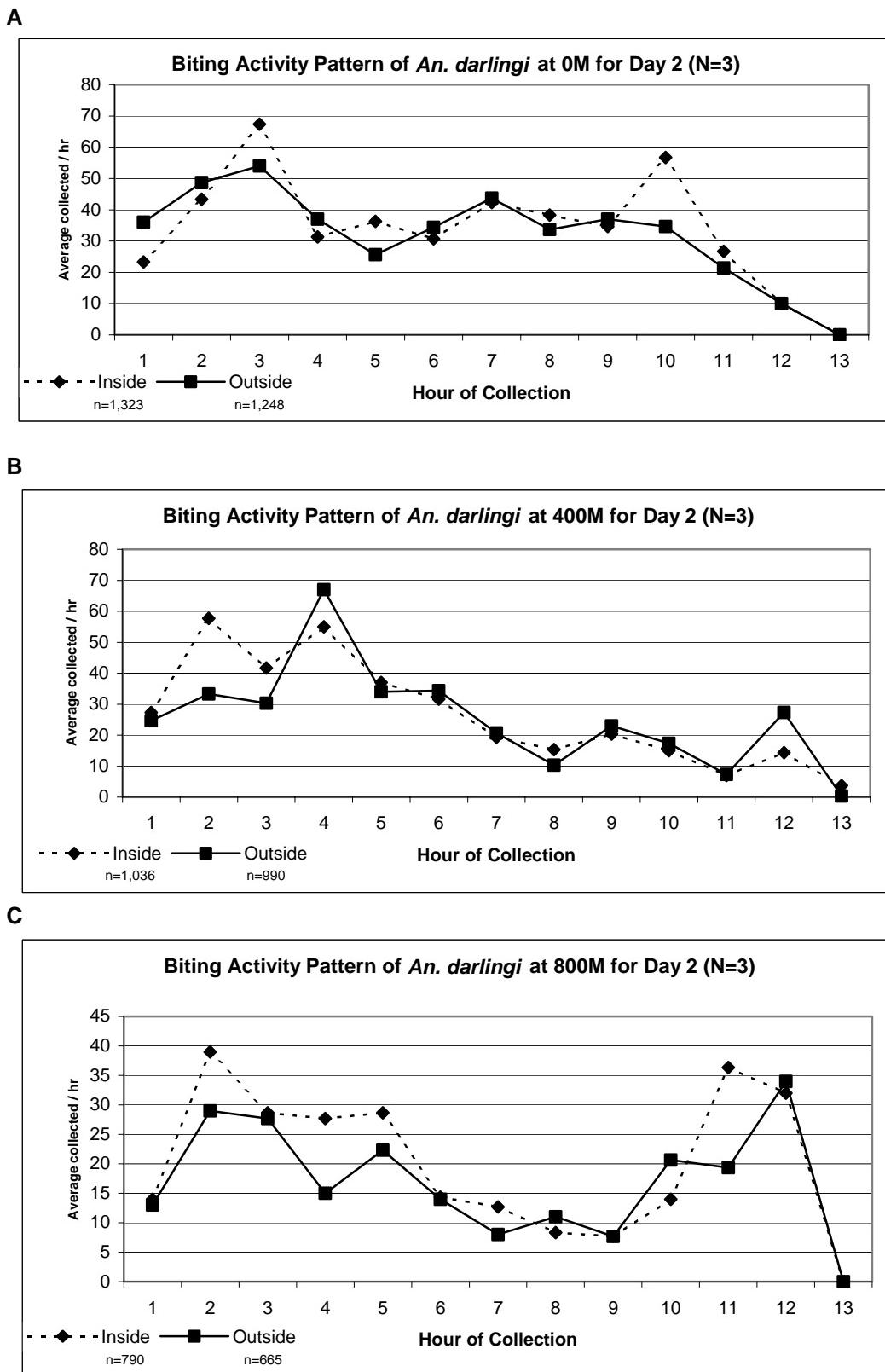


Figure 10. Biting activity patterns of unmarked *An. darlingi* females captured on Day 2 post-release at either (A) 0 M, (B) 400 M, or (C) 800 M from a fixed release site during July 2002-May 2003.

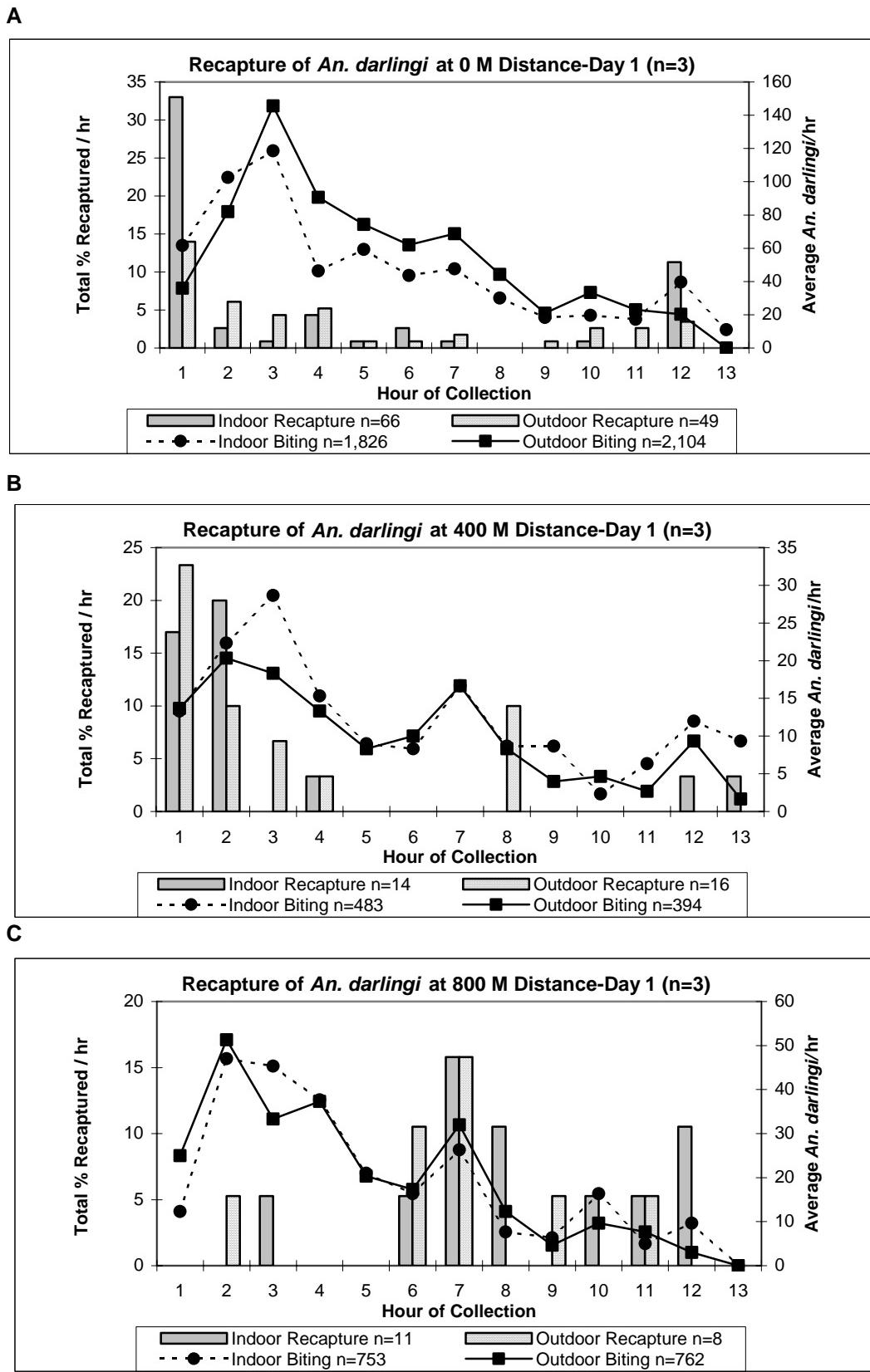


Figure 11. Hourly biting activity patterns of unmarked *An. darlingi* females overlaid onto the total percent recapture of marked specimens for collections performed on Day 1 post-release at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

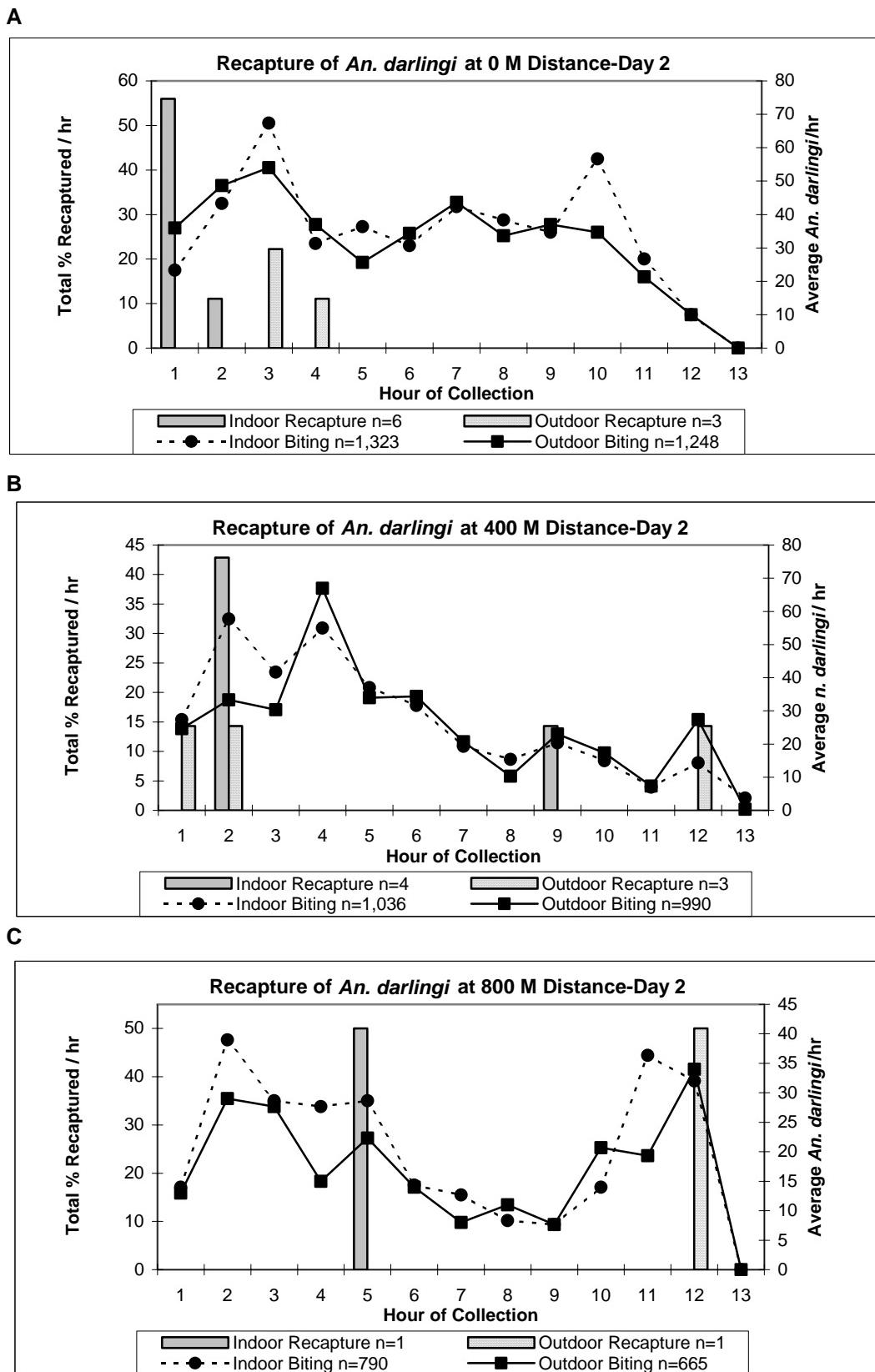


Figure 12. Hourly biting activity patterns of unmarked *An. darlingi* females overlaid onto the total percent recapture of marked specimens for collections performed on Day 2 post-release at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

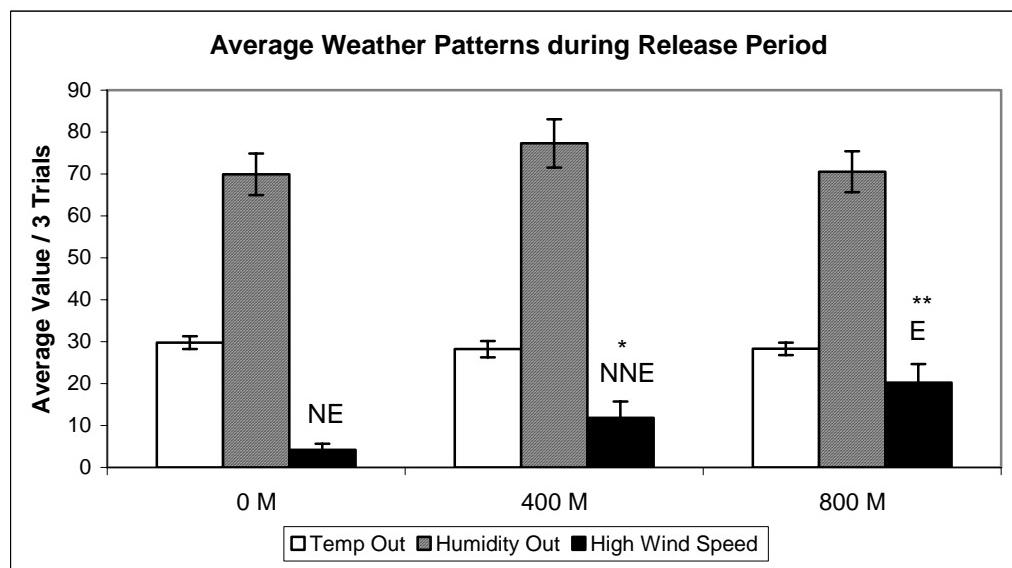
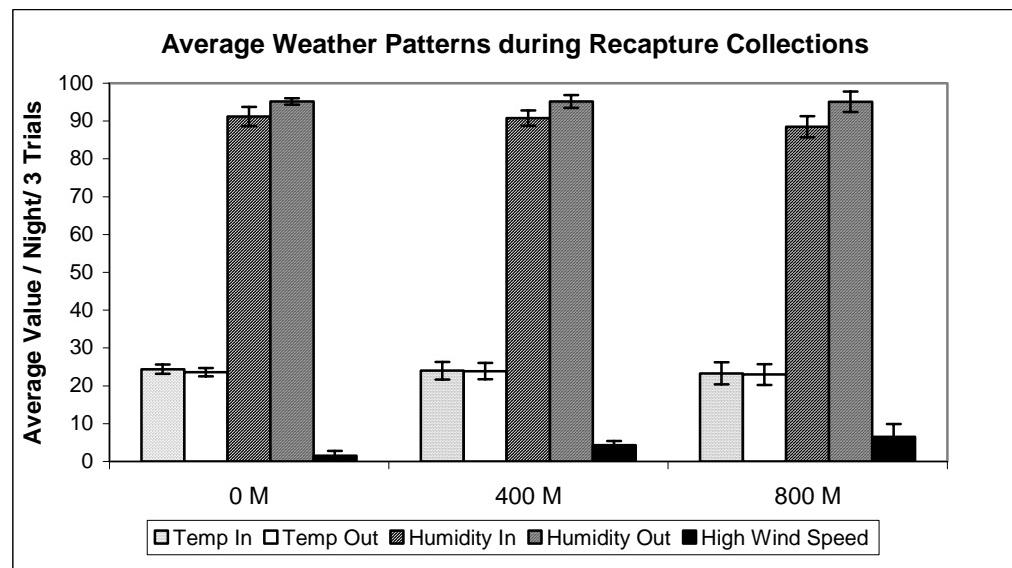
A**B**

Figure 13. (A) Average temperature (Celsius), relative humidity and maximum wind speeds (km/hr) of all-night recapture collections at either 0 M, 400 M or 800 M from a fixed release point. (B) Average environmental data for the hour between the release of marked *An. darlingi* specimens and the beginning of the recapture collections. Wind direction is depicted above the corresponding bars. NE=Northeast; NNE=North, Northeast; E=East. * and ** designate significances.

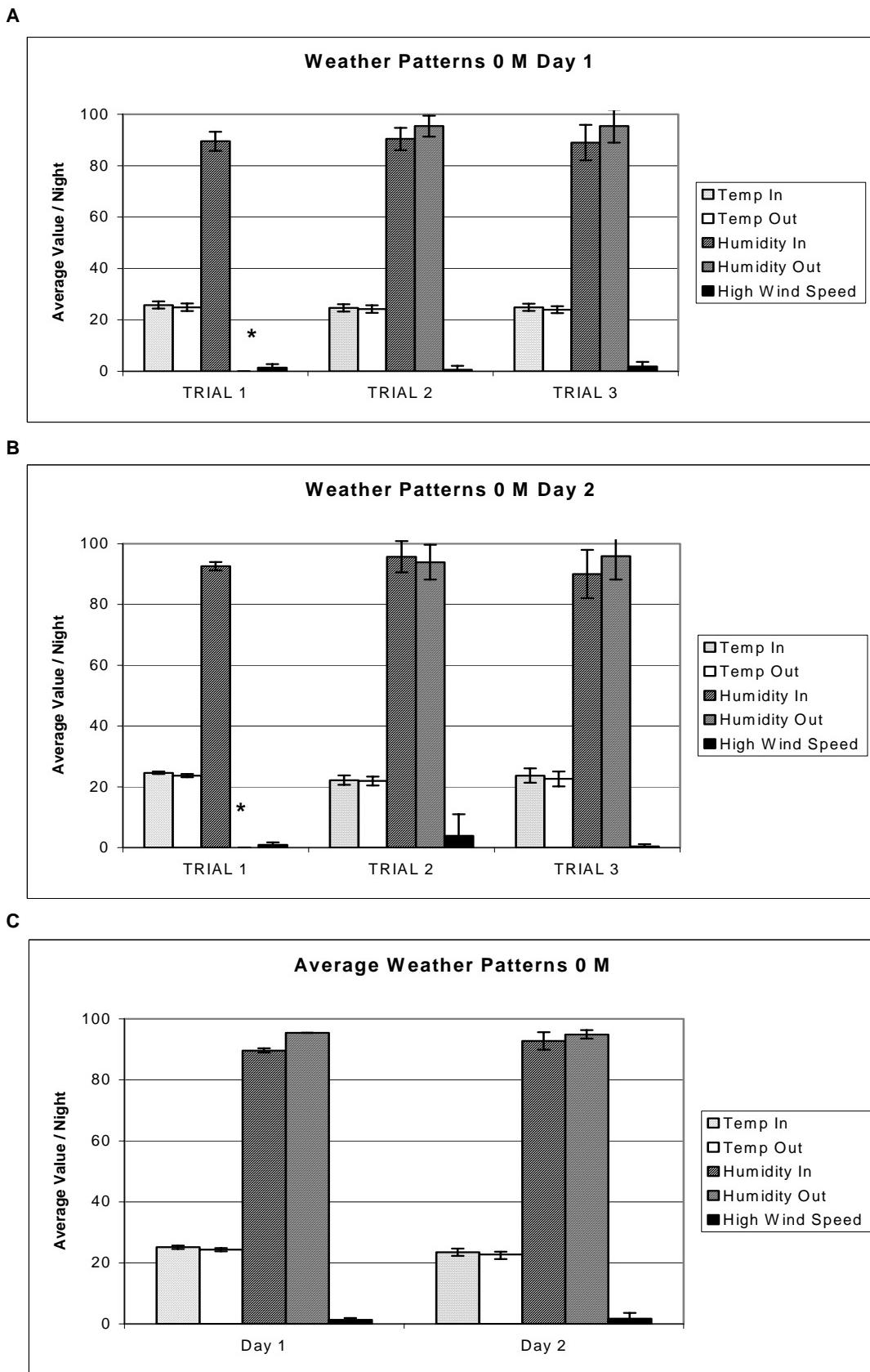


Figure 14. Average values at the 0 M site for indoor and outdoor temperatures (Celsius), relative humidity levels and high wind speed (km/hr) for individual sampling trials on (A) Day 1 and (B) Day 2 post-release. (C) The average weather pattern for all three trials conducted on either Day 1 or Day 2. * Data unavailable due to malfunction of weather station.

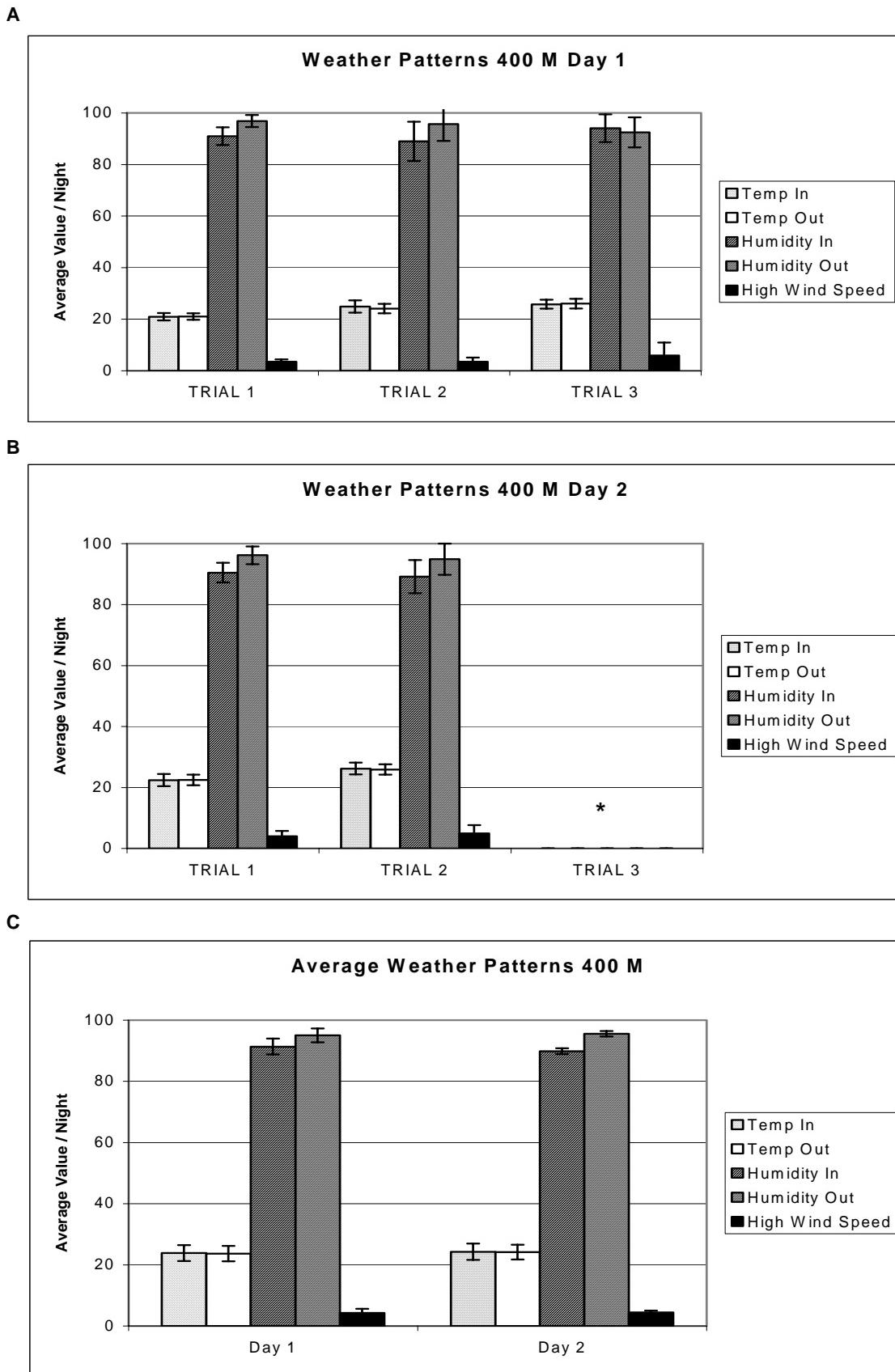


Figure 15. Average values at the 400 M site for indoor and outdoor temperatures (Celsius), relative humidity levels and high wind speed (km/hr) for individual sampling trials on (A) Day 1 and (B) Day 2 post-release. (C) The average weather pattern for all three trials conducted on either Day 1 or Day 2. * Data unavailable due to malfunction of weather station.

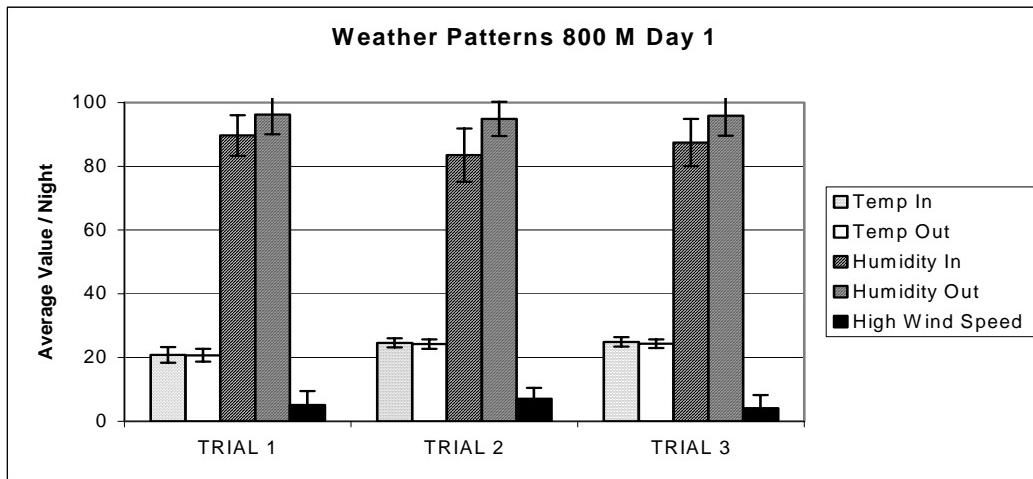
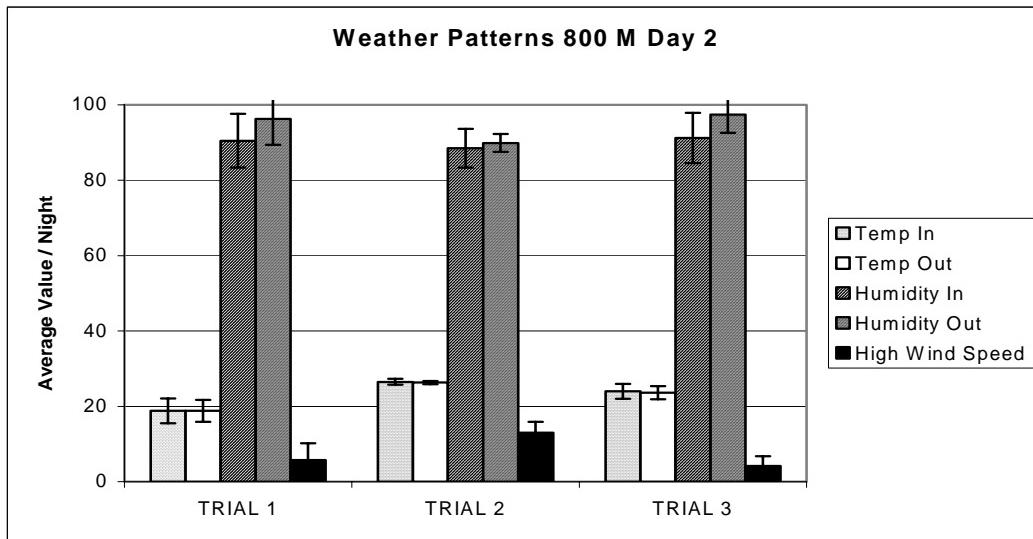
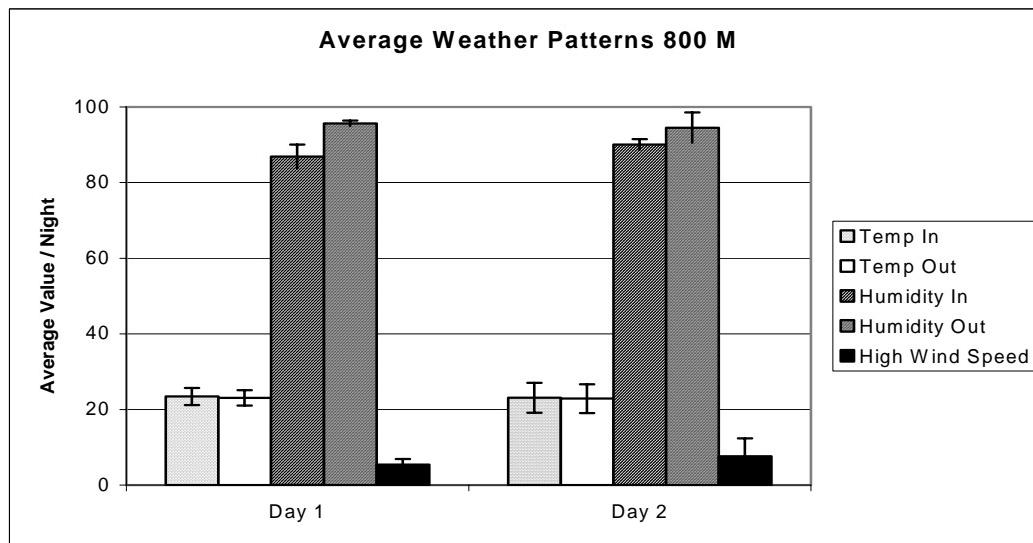
A**B****C**

Figure 16. Average values at the 800 M site for indoor and outdoor temperatures (Celsius), relative humidity levels and high wind speed (km/hr) for individual sampling trials on (A) Day 1 and (B) Day 2 post-release. (C) The average weather pattern for all three trials conducted on either Day 1 or Day 2.

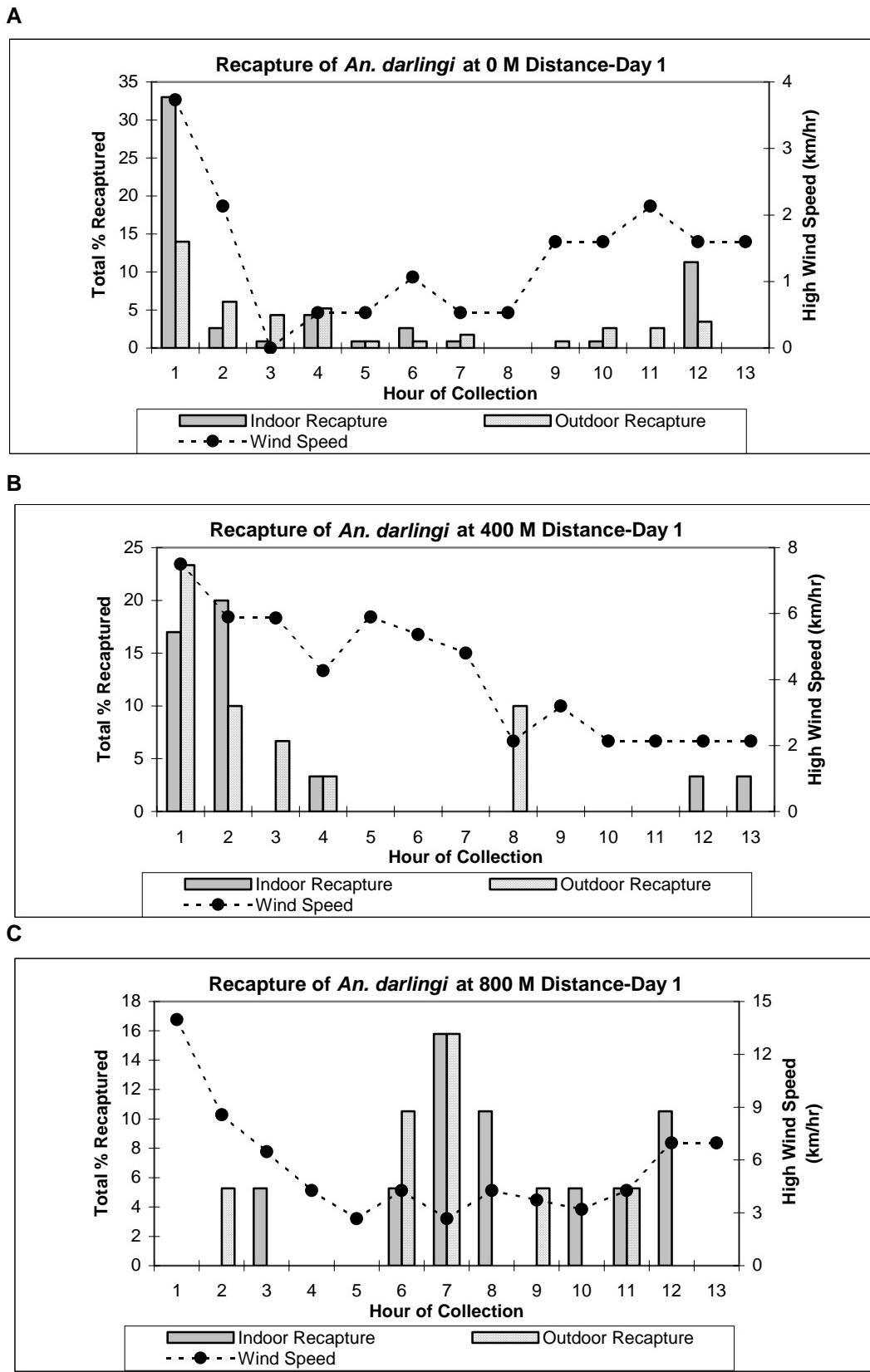


Figure 19. Day 1 average wind speed patterns overlaid onto the total % recapture of marked *An. darlingi* females by collection hour at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

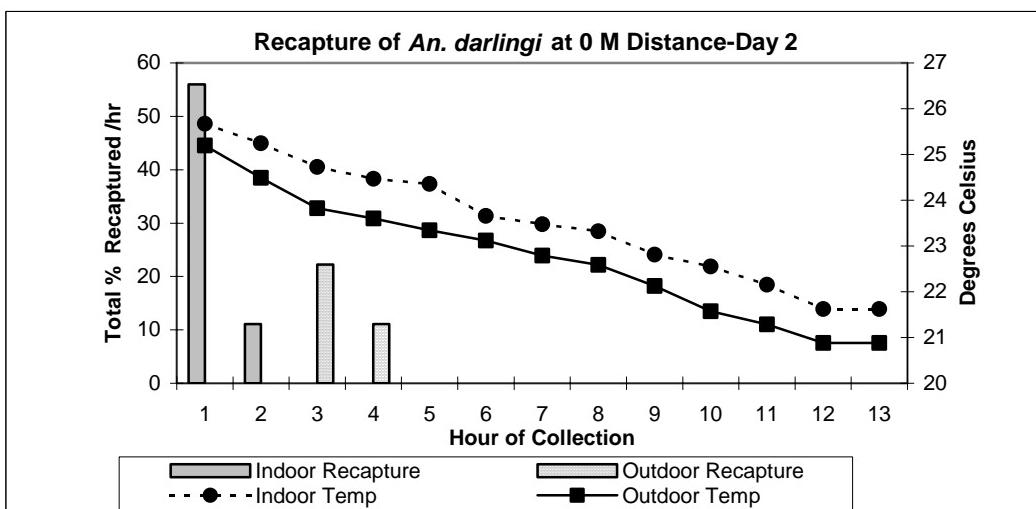
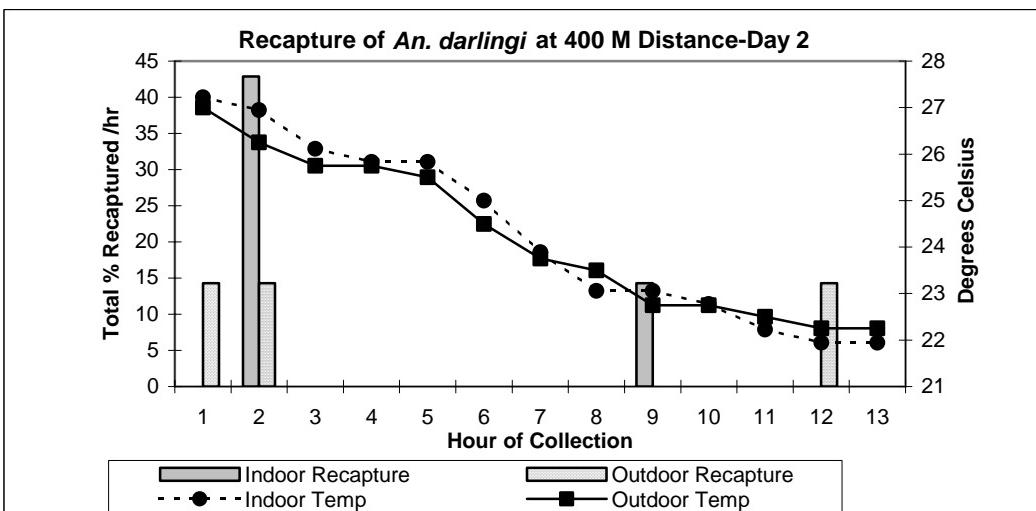
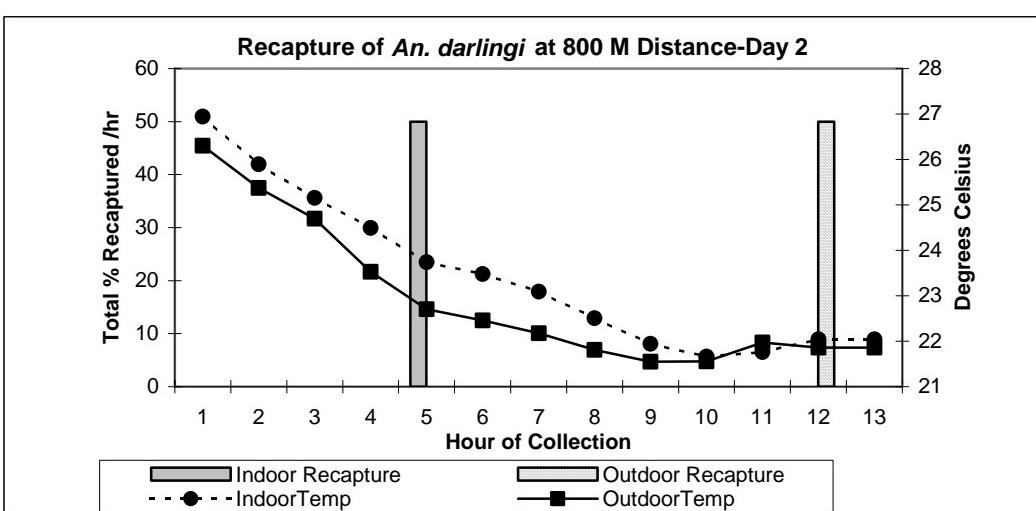
A**B****C**

Figure 20. Day 2 indoor and outdoor average temperature patterns overlaid onto the total % recapture of marked *An. darlingi* females by collection hour at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

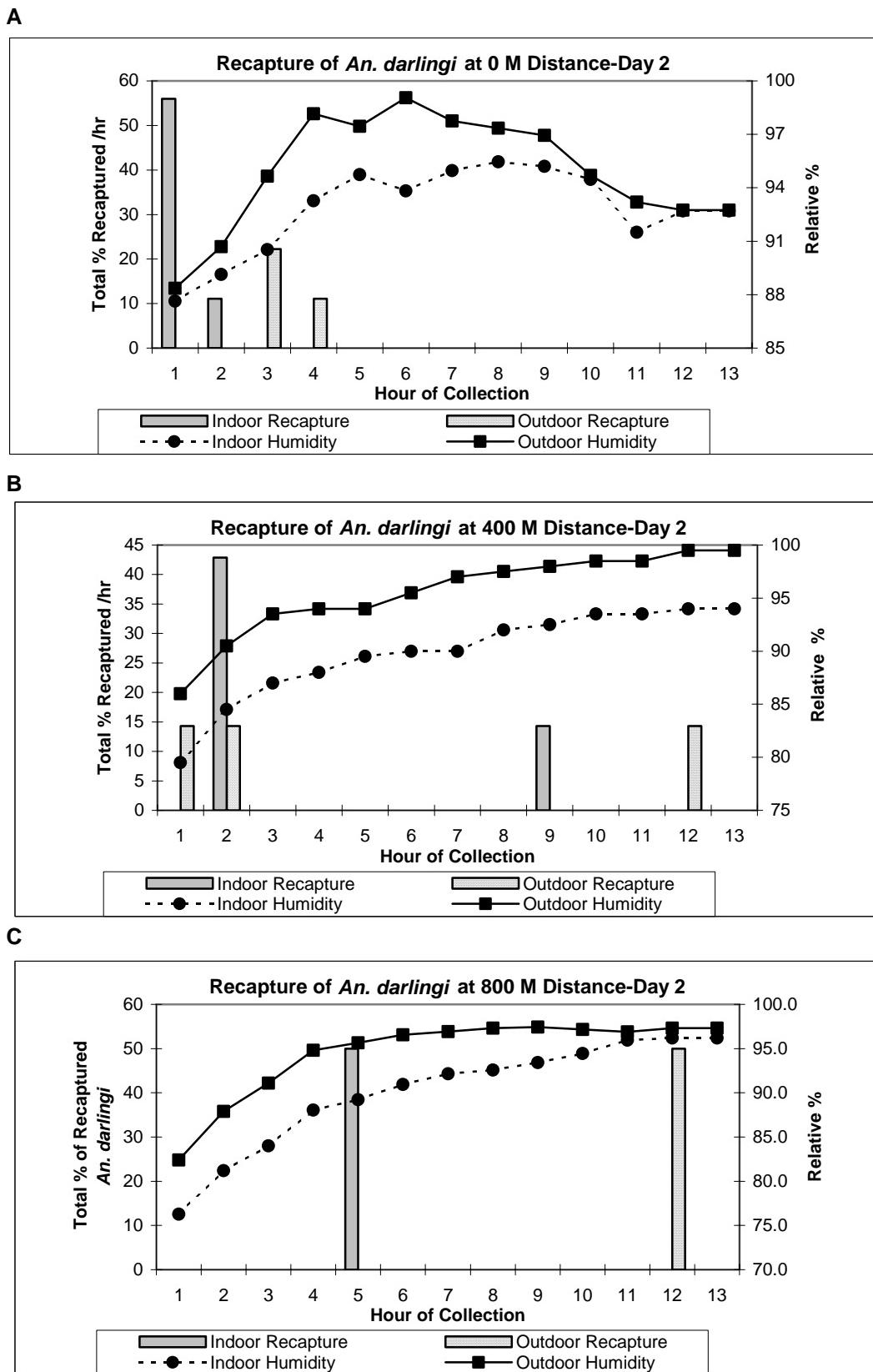


Figure 21. Day 2 indoor and outdoor average humidity patterns overlaid onto the total % recapture of marked *An. darlingi* females by collection hour at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

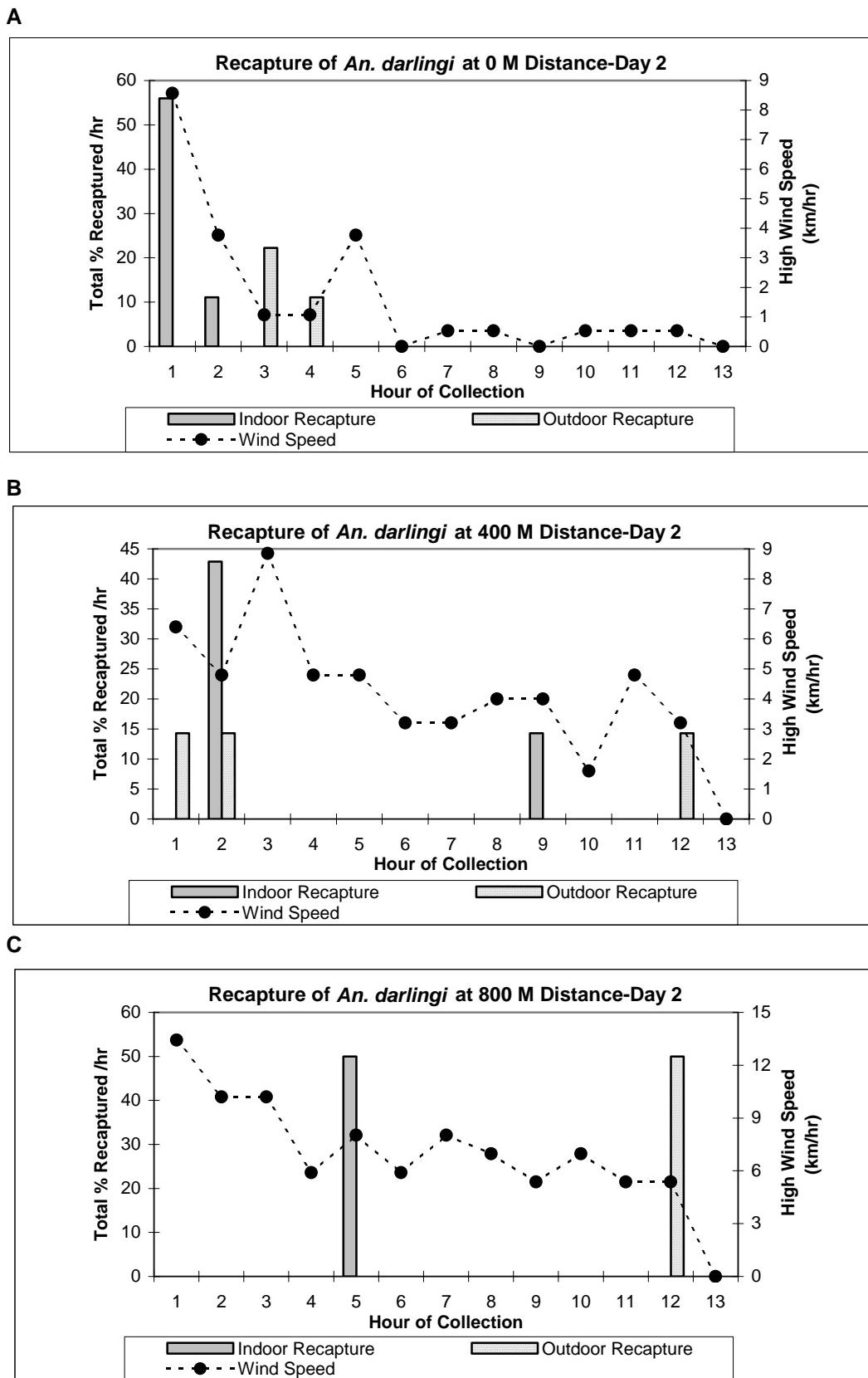


Figure 22. Day 2 average wind speed patterns overlaid onto the total % recapture of marked *An. darlingi* females by collection hour at either (A) 0 M, (B) 400 M or (C) 800 M from a fixed release point.

Table 1. Results from 18 all-night biting collections conducted from July 2002-May 2003 as part of a mark-release-recapture study of *An. darlingi* in Belize, Central America.

Species	Total Collected	Indoor	Outdoor	I:O
<i>An. darlingi</i>	12,376	6,212	6,164	1.00:0.99
<i>An. albimanus</i>	409	196	213	1.00:1.08
<i>An. pseudopunctipennis</i>	371	242	129	1.00:0.53
<i>An. punctimacula</i>	28	15	13	1.00:0.86
<i>An. vestitipennis</i>	17	8	9	1.00:1.13
<i>An. apicimacula</i>	1	1	0	-
Aberrant <i>An. darlingi</i> ^a	29	12	17	1.00:1.42

^aHarbach et al. 1993

Distance	Trial	Collection Night	# <i>An. darlingi</i> Recaptured	Total In	Total Out
0 METER Release=428 Recapture Rate 29.0%	A Release=144	1	28	14	14
		2	6	6	0
		Total	34	20	14
	B Release=175	1	65	43	22
		2	2	0	2
		Total	67	43	24
	C Release=109	1	22	9	13
		2	1	0	1
		Total	23	9	14
	Total		124	72	52
400 METER Release=396 Recapture Rate 11.6% ^a	A Release=174 (96) ^b	1	10	5	5
		2	4	3	1
		Total	14	8	6
	B Release=78	1	8	4	4
		2	1	0	1
		Total	9	4	5
	C Release=144	1	12	5	7
		2	2	1	1
		Total	14	6	8
	Total		37	18	19
800 METER Release=361 Recapture Rate 5.82%	A Release=54	1	2	0	2
		2	0	0	0
		Total	2	0	2
	B Release=203	1	13	8	5
		2	1	1	0
		Total	14	9	5
	C Release=104	1	4	3	1
		2	1	0	1
		Total	5	3	2
	Total		21	12	9

^aRecapture rate (37/318) after calculating a 45% mortality (i.e., 396-78=318) occurring in the first sampling trial (see footnote below).

^bThe release value of 96 reflects a 45% (9/20) mortality rate occurring in the control population.

Table 2. Results from 18 all-night paired human-baited biting collections conducted in a flight distance study to capture marked *An. darlingi* females. The number recaptured are shown according to distance, trial, day of collection and hut station (i.e., indoor and outdoor). The total number released from all three trials is listed below each distance.

Table 3. Results from 18 all-night paired human-baited biting collections used in a flight distance study of female *An. darlingi*. The number of marked specimens recaptured are shown according to distance, day of collection, time period and hut station (i.e., indoor or outside).

Distance	Collection Day	# <i>An. darlingi</i> recaptured	Hours 1-4 In Out		Hours 5-9 In Out		Hours 10-13 In Out	
0 METER Released=428	DAY 1 N=3	115 (93%)	47 (41%)	34 (30%)	5 (4%)	4 (3%)	14 (12%)	11 (10%)
	DAY 2 N=3	9 (7%)	6 (67%)	3 (33%)	0	0	0	0
		Total: 124	43%	30%	4%	3%	11%	9%
			73%		7%		20%	
400 METER Released=396	DAY 1 N=3	30 (81%)	12 (40%)	13 (43%)	0	3 (10%)	2 (7%)	0
	DAY 2 N=3	7 (19%)	3 (43%)	2 (29%)	0	0	1 (14%)	1 (14%)
		Total: 37	41%	41%	-	8%	8%	3%
			81%			8%		11%
800 METER Released=361	DAY 1 N=3	19 (90%)	1 (5%)	1 (5%)	6 (32%)	5 (26%)	4 (21%)	2 (11%)
	DAY 2 N=3	2 (10%)	0	0	1 (50%)	0	0	1 (50%)
		Total: 21	5%	5%	33%	24%	19%	14%
			10%		57%		33%	

Chapter 4

An evaluation of overhanging bamboo as a selection criterion for *Anopheles darlingi* larval breeding habitats in Belize, Central America

ABSTRACT

Previous studies in Belize have shown the preferred breeding habitats of the malaria vector *Anopheles darlingi* to be floating detritus patches associated with overhanging bamboo within riverine systems. This study evaluated the importance of overhanging bamboo as a mechanism for habitat selection. Four sets of 1-m² enclosure traps were placed into a fresh-water riverine system at a location known to harbor larval and adult *An. darlingi* populations. Each trap set comprised a control treatment (i.e., open water) and three experimental treatments consisting of detritus, detritus with overhanging bamboo, and overhanging bamboo alone. Larvae were sampled from all treatments within each set of traps on Day 5, Day 11 and Day 17 post-setup. Upon completion of the last sampling day, treatment positions were rotated within each trap set and new treatment material applied, which designated the beginning of the next sampling period.

A total of 2,461 *An. darlingi* larvae were collected and identified from twelve replicates conducted from March-May 2002. Of these, 1,997 larvae were sampled from detritus treatment traps, 256 from traps with bamboo and detritus; 139 from the bamboo treatment; and 69 from control traps. Overall, first instar larvae were the most abundant stage collected from all treatments. ANOVA statistics indicate the detritus treatment had a significantly higher average number of *An. darlingi* larvae than the other traps ($F=18.6$; $p<0.0001$), and that no difference existed between the control treatment and treatments containing either detritus with overhanging bamboo ($p=0.751$) or overhanging bamboo alone ($p=0.996$). These data suggest that overhanging bamboo does not contribute to the attractiveness of detritus material as an *An. darlingi* larval habitat, but acts as a barrier to surface flow of river water causing the lodging of debris that may already contain larvae.

In addition, the accumulation of detritus will attract gravid *An. darlingi* females for oviposition.

INTRODUCTION

Mosquitoes can be found breeding in a wide variety of terrestrial water accumulations; however, individual species are adapted to particular types of habitats. This restriction of a given species to a certain type of larval habitat is a result of selective oviposition by the adult female mosquito (Wallis 1954). However, the method of site selection is extremely complex and unknown for many vector species. Studies on the influence of water vapor, temperature, vision, background color, preoviposition behavior, osmotic pressure, inorganic compounds and physical obstruction have been reviewed by Clements (1999).

A primary vector of malaria in Central and South America (Forattini 1962), *An. darlingi* is defined as a riparian mosquito because of its association with river habitats throughout its geographic distribution (Faran and Linthicum 1981). As one of the anophelines of special interest in Belize, surprisingly limited studies have been conducted on the larval ecology of *An. darlingi* (Harbach et al. 1994; Rejmankova et al. 2000) since its first discovery in the 1940's (Komp 1940; Kumm and Ram 1941). Manguin et al. (1996) reported that the ecological determinants of *An. darlingi* larval habitats included floating mats of detritus in shaded areas along freshwater river margins with submersed plants. In particular, the floating mats were typically present in locations containing overhanging spiny bamboo (*Guada longifolia*) along the riverbank. Research on *An. darlingi* larval ecology from South America have also reported breeding sites to include

shaded areas of debris and floating vegetation in rivers where the flow of surface water is impeded (Fleming 1963; Panday 1980; Hudson, 1984).

Despite its importance in larval vector control, habitat selection by malaria vectors is a poorly understood ecological process (Krebs 1994). Buxton and Hopkins (1927) recognized both intrinsic and extrinsic factors associated with breeding sites that influence egg laying. Intrinsic factors include water temperature, salinity, pH and microorganisms. Extrinsic factors include light intensity, environmental variables, and landing sources to include surrounding vegetation. Because most mosquito breeding is associated with aquatic vegetation (Bates 1949), an understanding of the plant-vector relationship is necessary for proper control and management techniques.

The influence of plants is important to both adult and immature mosquitoes. Aquatic vegetation may contribute to the success of immature stages by reducing wave action of the surface water and thus provide a more stable interface for the exchange of gases (Rueger et al. 1964) and provide shade from solar radiation (Zetek, 1920). In addition, plants within breeding habitats can act as a substrate for algae and other larval food (Barber and Hayne 1925) and protect against predation (Orr and Resh 1989). The effect of plant manipulation on larval survival and densities has been previously examined. Orr and Resh (1990) demonstrated that mechanical harvesting of parrotfeather (*Myriophyllum aquaticum*) in a freshwater marsh reduced *Anopheles* spp. abundance. Data from Belize indicate removal of *Typha domingensis* from macrophyte marshes will decrease densities of *An. vestitipennis* larvae but cause an increase in *An. albimanus* larvae (Grieco pers. comm.).

Vegetation may also influence adult mosquitoes by physically restricting oviposition due to disrupting the behavior of the female or rendering the surface of the water inaccessible (Macan and Worthington 1951). On the other hand, metabolites or biochemical processes of plants may act as attractants to the female, leading her to favorable oviposition habitats (Rapp and Emil 1965). Field research in Belize has shown selective oviposition of *An. albimanus* females within enclosure areas containing cyanobacterial mats compared to those with only open water (Rejmankova et al. 1996). In addition, laboratory studies indicate the preference of gravid *An. albimanus* females to oviposit within beakers containing volatiles from these cyanobacterial mats (Rejmankova et al., pers. comm.). Similar results are being reported using *An. vestitipennis* females and volatiles from their corresponding breeding sites (Rejmankova et al., pers.comm.)

The objective of the present study was to determine the importance of overhanging bamboo as an *An. darlingi* larval habitat selection criterion under field conditions. Understanding the degree to which this vegetation type is used in the selection process of gravid *An. darlingi* can be useful in decision-making processes involving larval management and control.

MATERIALS AND METHODS

Study Site: Based upon preliminary surveys that established locations of larval and adult *An. darlingi* populations (Appendix I and VII), enclosure traps were placed into St. Thomas Creek located at N17°08'59.9" and W88°37'46.8" in the central Cayo District of Belize, Central America (Figure 1). Positioned at the foothills of the Maya Mountains, temperatures range from 29°C in January to 34°C in May. Annual rainfall at the site for the year of the study totaled 3,237 mm with a distinct dry season occurring from

February-May (419 mm) and a rainy season occurring from June-January (2,818 mm).

The study site is in the mid-reaches of the Sibun River Watershed. The input of several tributaries has made this area prone to frequent flooding which creates an alluvial plain ideal for bamboo growth.

Enclosure Traps: Sixteen 1 m x 1 m x 25.4 cm bottomless, screened enclosure traps were constructed using locally acquired materials (Figure 2). Each trap consisted of four individual 1 m x 25.4 cm screened panels fastened at the corners using hinges. Panels were designed after window screens and were constructed using 1 in. aluminum framing joined by L-brackets. Panel framework was fitted with 1/16 in. plastic mesh screening using rubber-sealing cord. Empty 1-liter water bottles were used as flotation devices and fastened to all four corners of each trap using tie wire. Traps were floated in the water such that the top of the enclosure was approximately 4 in. above the surface. Traps were held in position by suspending nylon rope weighted with riverbed rocks through each of the corners and suspending them from an overhanging ½” mesh sheet. This ensured positional stability but allowed the enclosures to rise and fall with fluctuating water levels. The mesh sheet also provided a medium from which the bamboo could be suspended and minimized the accumulation of falling detritus from the stream bank into the traps.

Treatments: A total of four sets of traps, each containing four treatments, were used in the study. This resulted in four replicates being run simultaneously during one sampling period. Treatments included: 1) open water as a control; 2) detritus; 3) detritus with overhanging bamboo; and 4) overhanging bamboo alone. The detritus treatment consisted of dried twigs and sticks collected from the upper banks of the stream margin to cover the surface of the water in the corresponding trap. Prior to the beginning of the study a

sample of detritus material was placed into rearing pans containing stream water to determine the potential for dormant viable eggs. No larvae were produced from three of these trials. Overhanging bamboo treatment comprised an average of twenty-3 ft. sections of fresh *Guada longifolia* cut from the stream margin. Each bamboo piece was inverted and tied to the suspended mesh sheet over the corresponding trap using nylon rope such that the leaves touched and covered the surface of the water. This mimicked natural conditions observed during preliminary surveys. In combined detritus and overhanging bamboo treatments, the detritus was placed into the trap before hanging the bamboo.

Mosquito Sampling: Anopheline larvae were sampled from each enclosure trap on Day 5, Day 11 and Day 17 post-setup using a standard larval dipper. Collectors were rotated between treatments and trap sets during each sampling period. All anopheline larvae collected from 30 dips were placed into individually labeled 4 oz. Whirl-Pak® bags (Nasco, Ft. Atkinson WI) and later identified to species (Clark-Gil and Darsie 1983). An even distribution of larval sampling within enclosure traps was obtained by visually separating the trap into quadrants, and taking equal dips without contacting the screen surface. On the last day of sampling, treatment positions were rotated within each trap set to prevent positional biases and new treatment material applied. The number of larvae by instar, species, treatment and sampling day was recorded (Appendix VIII) and later analyzed using SPSS (version 9.0, SPSS Inc.) statistical software.

RESULTS

Three sampling periods, each with a maximum of 12 treatment replicates, were conducted from March-May 2002. A total of 3,182 anopheline larvae were collected including: 2,461 *An. darlingi* Root; 540 *An. albimanus* Wiedemann, 175 *An. vestitipennis*

Dyar and Knab/*An. punctimacula* Dyar and Knab and 2 *An. gabaldoni* Vargas (Table 1).

In addition, 4 *Chagasia bathana* Dyar were also found in the enclosure traps. Because of the extreme difficulty in distinguishing larvae of *An. vestitipennis* from *An. punctimacula* in 1st-3rd instar stages (Jim Pecor WRBSU, pers. comm.), the investigator did not attempt separation of these species. For this reason, these species were grouped with *An. gabaldoni* and *Chagasia bathana* and classified as “other” species during descriptive analyses. The ease of identifying *An. albimanus* larvae at all instars permitted the separation of this species from other non-target species (Table 1).

During the study period, water height within the adjacent Sibun River ranged from 61 cm in February to 41 cm in April and little precipitation was recorded with only 2 mm falling in February, 3 mm in March and none in April. Water levels at the St. Thomas creek site fluctuated within only 1 cm averaging 60 cm at all trap locations. Water temperatures consistently averaged 25°C at each trap set site during all three sampling periods. The average high temperature ranged from 29°C in February to 33°C in April. Low temperatures averaged 20°C.

Overall, the majority of all larvae (78.2%; 2,490/3,182) were collected from enclosure traps containing the detritus treatment (Table 1). Bamboo with detritus treatments contained the next largest larval population (338/3,182), while traps with bamboo alone only comprised 7.0% (224/3,182) of all larvae sampled. Control traps contained the smallest larval population (4%; 130/3,182). Examining data for the target species, the majority (81%; 1,997/2,461) of all *An. darlingi* larvae (i.e., 1st-4th instar) were collected from traps containing the detritus treatment (Table 1). Control traps contained only 2.8% (69/2,461) of the total *An. darlingi* larvae collected, while 256

(10.4%) were collected from detritus with overhanging bamboo traps, and 139 (5.6%) were collected from traps containing overhanging bamboo alone. These same trends occurred in the combined “other” group of larval species collected at the study site (Table 1). Results of *An. albimanus* larval populations show 77.7% (420/540) of all larvae sampled were collected from detritus traps, and only 5.0% (27/540) were sampled from the control treatment (Table 1). Unlike that seen for the target species, however, samples from traps containing overhanging bamboo alone produced slightly more (10.9%; 59/540) of the total *An. albimanus* larval population compared to the 6.2% (34/540) collected from the bamboo and detritus combined treatment.

Statistical analyses of the target species indicate a significant difference in the total average number of *An. darlingi* larvae (i.e., 1st-4th instar) collected from enclosure traps containing detritus treatments compared to the other three treatments (ANOVA; F=18.6 p<0.0001). No difference was found between the control treatment and traps containing both overhanging bamboo with detritus (p=0.751) or overhanging bamboo alone (p=0.966).

The majority of all sampled larvae (75.0%; 2,380/3,182) were of the 1st instar (Table 1). Second instars comprised 28.3% (903/3,182), and those larvae in the 3rd and 4th instar represented only 5% (155/3,182) and 3% (97/3,182) of the total population, respectively. Similar trends were seen within individual larval populations of *An. albimanus* and other species. Analyses of the sampled *An. darlingi* populations show the overall average number of 1st instar larvae to be significantly greater (ANOVA; F=18.0, p=<0.0001) than 2nd, 3rd, and 4th instar populations.

Examination of the average number of individual *An. darlingi* instar populations among treatments showed significantly more 1st (ANOVA; F=12.1, p=<0.0001) and 2nd (ANOVA; F=10.5, p=<0.0001) instar larvae were collected in detritus traps compared to traps containing other treatments (Table 1). The average number of 3rd instar *An. darlingi* larvae sampled from the detritus traps was significantly greater (ANOVA; F=5.706, p=0.002) than those collected from traps with open water (p=0.003) and overhanging bamboo treatment alone (p=0.027). However, no difference was seen in the number of 3rd stage larvae collected from traps with detritus compared to the numbers collected from overhanging bamboo with detritus treatments (p=0.713). In addition, no difference was indicated in the number of 4th instar *An. darlingi* larvae collected among treatments (ANOVA; F=2.270, p=0.095).

The total number of *An. darlingi* larvae collected from all treatments on Day 5 post-setup (897) was higher than that sampled on Day 11 (822) or Day 17 (743) (Table 2). However, there was no significant difference in the total average number of larvae collected among sampling days (ANOVA; F=0.059, p=0.943). Detailed examinations were conducted to determine if differences in *An. darlingi* larval populations existed over time within individual treatments. This data could be used to describe the suitability of a particular treatment.

Within the traps containing open water, no significant difference was found among the average number of *An. darlingi* larvae collected on Day 5, Day 11 or Day 17 post-setup (ANOVA; F=0.714, p=0.492) (Table 2). In addition, when the number of each instar population was compared over time, no differences were indicated in the number of 1st instar (ANOVA; F=0.877, p=0.427), 2nd instar (ANOVA; F=1.272, p=0.295), 3rd

(ANOVA; $F=2.060$, $p=0.146$) or 4th instar (ANOVA; $F=1.233$, $p=0.306$) larvae among Day 5, Day 7 or Day 17 post-setup.

Similar results were seen from detritus treatments where no differences existed in the average larval density among the three sampling days post-setup (ANOVA; $F=0.045$, $p=0.956$) (Table 2). Again, when the number of each larval instar population was analyzed between Day 5, Day 11 and Day 17, no significant changes were seen in the 1st instar (ANOVA; $F=0.849$, $p=0.438$), 3rd instar (ANOVA; $F=2.116$, $p=0.139$) or 4th instar (ANOVA; $F=1.499$, $p=0.306$) populations. However, a significant increase (ANOVA; $F=7.996$, $p=0.002$) was indicated in the number of 2nd instar larvae sampled from Day 17 compared to Day 11 ($p=0.028$) and Day 5 ($p=0.001$). No difference existed between sampling Day 5 and Day 11 ($p=0.539$).

For the enclosure traps containing only overhanging bamboo, the overall average larval population densities did not change over sampling days (ANOVA; $F=1.044$, $p=0.355$) (Table 2). As with the control traps, no significant differences were seen over sampling days for any of the instar populations (ANOVA 1st instar; $F=1.325$, $p=0.281$ / ANOVA 2nd instar; $F=2.416$, $p=0.107$ / ANOVA 3rd instar; $F=0.219$, $p=0.804$ / ANOVA 4th instar; $F=0.181$, $p=0.835$).

Data from overhanging bamboo and detritus treatments show a significant increase in the number of *An. darlingi* larvae collected from Day 5 sampling versus Day 11 sampling (ANOVA; $F=3.701$, $p=0.029$) (Table 2). However, no significant changes were seen between Day 11 and Day 17 sampling ($p=0.547$). Among each of the sampling days, no difference was indicated in the number of 1st instar (ANOVA; $F=2.834$,

$p=0.087$), 2nd instar (ANOVA; $F=3.263$, $p=0.063$), 3rd (ANOVA; $F=1.020$, $p=0.382$) or 4th instar (ANOVA; $F=2.427$, $p=0.118$) larval populations sampled over time.

Comparisons of individual larval instar population densities were also conducted between treatments for each sampling day (Table 2). The results revealed a significantly higher mean for 1st instar populations within the detritus traps compared to other treatments at Day 5 (ANOVA; $F=6.38$ $p=0.001$), Day 11 (ANOVA; $F=10.53$ $p<0.001$) and Day 17 (ANOVA; $F=7.67$ $p=0.001$). No other differences were seen within 2nd-4th instar populations among treatment types for Day 5. At Day 11, the detritus treatment now also contained higher numbers of 2nd instar larvae than the control traps ($p=0.048$) but were not significantly different than the 2nd instar populations within the other treatments. No differences were found within 3rd stage average larval populations (ANOVA; $F=2.980$, $p=0.046$). The overhanging bamboo with detritus treatment had a significantly greater average of 4th instar larvae than the control traps (ANOVA; $F=3.439$, $p=0.045$) and treatments consisting of overhanging bamboo alone ($p=0.026$), but the density was no different compared to traps containing detritus treatments ($p=0.200$). Analyses of data from Day 17 sampling, in addition to a greater 1st instar larval population (see above), indicate the average population density of 2nd instar larvae in the detritus trap was greater than the other three treatment types (ANOVA; $F=13.54$ $p=0.001$), but no other differences in the average number of stage 3 or stage 4 larvae was seen.

DISCUSSION

There are several beneficial effects of vegetation on *Anopheles* larval densities including protection from predators and physical disturbance, food resources, favorable

oviposition substrate and optimal thermal conditions for rapid larval development (see references in Orr and Resh 1989; Collins et al. 1988). Experimental tests under field conditions can provide insight into these influences. The primary objective of the present study was to elucidate the role of overhanging bamboo in the habitat selection of *An. darlingi* in freshwater river systems in Belize.

Previous research in Belize has characterized *Anopheles darlingi* breeding habitats as floating mats of detritus in freshwater river systems (Komp 1941; Kumm and Ram 1941; Manguin et al. 1996). These mats contain debris including wood pieces, leaves and seeds from vegetation along the riverbanks and were found to be associated with spiny bamboo (*Guadua longifolia*) overhanging into the surface water (Manguin et al. 1996). Because of the importance of *An. darlingi* in malaria transmission (Deane et al. 1948; Foote and Cook 1959; Fleming 1986; Klein et al. 1991; Grieco 2001; Achee et al. 2000), a critical understanding of the importance of bamboo in the selection of larval breeding sites in Belize was crucially needed.

Overall, enclosure traps containing the detritus treatment produced the greatest *An. darlingi* larval populations. Although not a significant difference, traps containing the overhanging bamboo treatment produced more larvae than the control treatment. This suggests that the bamboo may be acting as a landing place for gravid *An. darlingi* females prior to egg laying. If pre-oviposition landing sites were important for gravid females, then traps containing the overhanging bamboo with detritus treatments would be expected to have similar if not greater larval populations as the traps with only detritus. This did not occur. However, there were more *An. darlingi* larvae sampled from traps

with both bamboo and detritus as compared to those with only bamboo further supporting the attractiveness of debris mats.

The size of the mesh screening used to construct the enclosure traps was specifically chosen to ensure that surface water flow within the traps was minimally affected. This mesh size could have allowed free-floating larvae to enter the enclosures by “drifting” between the openings. Rotating the upstream position of treatments between sampling periods controlled for potential larval drifting, and therefore accumulation, from one trap to another. Even when the overhanging bamboo treatment, which would provide a source of larval attachment, was in the first upstream position, more *An. darlingi* larvae were still found in the detritus traps further downstream (data not shown). However, data did indicate that drifting was a real event because, although densities were similar in all treatments, there were 3rd and 4th instar larvae sampled from traps on Day 5 post-setup. Under field conditions, the time period from 1st instar to pupal development for *An. darlingi* is approximately ten days (Grieco, pers. comm.) so the probability that 3rd and 4th stage larvae developed from a population of eggs oviposited within any of the traps is extremely small. Interestingly, Bruyning (1952) suggested that *An. darlingi* reinvades areas with temporary breeding habitats by downstream flotation of larvae between patches of water hyacinth along rivers in Surinam. No drifting *An. darlingi* larvae were collected in the present study when the area surrounding the traps was sampled but this technique was not systematic and future studies should incorporate an experimental design that quantifies the number of free-floating larvae.

Detailed examinations of each *An. darlingi* instar population by treatment type and sampling day provided further insight into both the attractiveness and suitability of

the particular habitat. The attractiveness of traps containing bamboo or bamboo with detritus to gravid *An. darlingi* females was no different than open water as defined by the number of 1st instar larvae on Day 5, Day 11 and Day 17 post-setup. However, those traps that contained only the detritus treatment were consistently more attractive to gravid *An. darlingi* females on all sampling days compared to the other treatments. The significantly greater number of 1st instar larvae in the detritus treatments at Day 5 compared to the other treatments further substantiates the attractiveness of this habitat. This is because under laboratory conditions the time period from oviposition to egg hatch is approximately 32-46 hrs. (Grieco, pers. comm.). In order to have such a large population of 1st stage larvae by Day 5, gravid females had to be attracted to the site within the first two days post-setup.

These results indicate factors intrinsic to the larval habitat may be affecting *An. darlingi* ovipositional behavior, including volatile substances from the detritus mats. Rejmankova et al. (2000) reported that larval habitats of *An. darlingi* had higher levels of bacteria compared to open water. These bacteria within the detritus traps could be acting as positive cues for oviposition. Other studies from Belize have reported that volatile substances from both *An. albimanus* and *An. vestitipennis* breeding sites act as species-specific ovipositional stimulants (Rejmankova et al. 1996; Grieco pers. comm.). Extensive research has shown the decomposition of certain organic matter to yield volatile chemicals that attract and induce gravid female mosquitoes to oviposit (Clements 1999).

Interestingly, the traps containing detritus with overhanging bamboo had significantly more 1st instar larvae than later stage instars on Day 5 and Day 11 post-setup

but were still not as attractive as traps with detritus alone. In addition, by Day 17 there was no difference between the densities of any of the larval instar populations in the traps containing overhanging bamboo and detritus. Because the type of detritus used was similar for both treatments, these data suggest potential influences from extrinsic factors related to the bamboo above the debris mat. First, the cut portion (i.e., stem) of the bamboo pieces could have emanated substances that confound the signature of volatiles released from the detritus material. This may have decreased the stimulatory effect for gravid *An. darlingi* females. Next, although the sampling period in the present study was dictated by the length of time the cut bamboo would remain green, some degradation of the bamboo leaves touching the surface water did begin to occur prior to fresh treatment. The biochemical process of this decay and the resulting metabolites may have had negative pre-ovipositional effects on gravid females by confounding or masking stimulatory volatiles emanating from the underlying detritus mat (Rapp and Emil 1965). De Zulueta (1950) reported that gravid *An. darlingi* females under laboratory conditions were repelled by infusions characterized by high organic content. Future habitat preference research of *An. darlingi* should attempt to isolate and quantify potential chemical cues from both positive and negative breeding habitats over time.

Another possible reason why the traps with overhanging bamboo and detritus did not produce similar numbers of *An. darlingi* larvae as those of detritus alone is that the bamboo could have created physical impedance that prevented some proportion of mosquitoes from reaching the debris mat. This would reduce the total number of eggs deposited. *Anopheles* mosquitoes lay their eggs singly on the surface of the water while either resting in contact with it or while flying over it (Clements 1999). Russell and Rao

(1940; 1942) reported the absence of *An. culicifacies* larvae in rice fields in India in response to mechanical obstruction of rice plants. Larvae were found during early stages of cultivation but disappeared when the plants reached a height of 0.3 m or more. When eggs of *An. culicifacies* were introduced into this rice-field water, the larvae hatched and underwent full development. When experimental pits were planted with rice seedlings 0.3-0.4 m in height and 0.1 m apart, the density of *An. culicifacies* larvae was greatly reduced compared to control pits.

Physical impedance created by the overhanging bamboo may also have negatively affected the pre-ovipositional behavior of *An. darlingi*, causing the cessation of the egg-laying process. The pre-ovipositional behavior of several mosquitoes has been described. Kennedy (1942) reported *Anopheles atroparvus* females were never seen to start an oviposition “hovering dance” without touching the water, and the females continued to touch the surface of the water intermittently during the dance. Bates (1940) stated that in a room-sized cage, the pre-ovipositional behavior of *Anopheles sacharori* consisted of short sidewise flights only a few centimeters above the water. From the same study, *Anopheles superpictus* females were seen to perform a series of up and down flights prior to ovipositing.

Another way in which overhanging bamboo could have physically affected gravid *An. darlingi* is by preventing the detection of the debris material in open water. The ability of pre-ovipositional females to distinguish between floating detritus mats from open water is based on specular reflectance, or the relative “lightness” of the water that occurs because of a surface sheen. Accumulated debris will appear darker than the surrounding water. Under laboratory conditions, *An. darlingi* laid more eggs in

oviposition dishes that had a dark bottom versus those with white bottoms (De Zulueta 1950). No information exists on the pre-ovipositional behavior of *An. darlingi* females in Belize. Further field and laboratory experimentation, in combination with volatile isolations, would prove useful in the understanding of habitat preference by gravid females of this important species.

If a breeding habitat was suitable for larval survival, a heterogeneous population of larval instars (i.e., 2nd, 3rd and 4th) may develop over time. This would reflect beneficial components such as proper food availability and/or protection from predators that enable the development of larvae. In the present study, the traps with the detritus treatment consistently produced the greatest number of 1st instar larvae on each of the sampling days post-setup; however, by Day 17, only the 2nd instar population had significantly increased within the detritus mats compared to other treatments. Unfortunately, bacterial abundance within enclosure traps was not measured during the study. Because of this, the quantification of change in food availability that might influence larval survival cannot be discussed. The most likely reason that the 3rd and 4th instar populations did not increase significantly over time in the detritus treatment is that large numbers of 1st instar larvae were removed within the habitat on the previous sampling day. This would reduce the total population available to develop into later instars, and would explain why the treatments with overhanging bamboo and detritus also did not produce high numbers of later stage larvae even though similar debris material was contained. Another explanation, although not as probable, could be due to larval movement from oviposition sites to adult emergence sites. Studies by Foley et al. (2002) have suggested this theory for *An. flavirostris* in the Philippines. Such information on *An.*

darlingi would be of great interest. Despite the small numbers of 3rd and 4th instar larvae in the detritus treatments, the significantly higher overall number of larvae sustained in the traps at Day 17 indicate the debris mats were the most suitable breeding site in the study.

In conclusion, an understanding of the mechanisms that produce preferred larval habitats for specific malaria vectors will allow more accurate predictions of anopheline population dynamics, and lead to the implementation of more effective control strategies. Data from the present study suggest that overhanging bamboo along river margins does not enhance the attractiveness of detritus mats to gravid *An. darlingi* females, but rather acts as an impedance to surface water flow and lodges floating debris that may already contain larvae. In addition, the lodged detritus material will then attract gravid females for oviposition. Further analyses are required to quantify the contribution of overhanging bamboo to the density of productive *An. darlingi* breeding sites along rivers in order to suggest management options.

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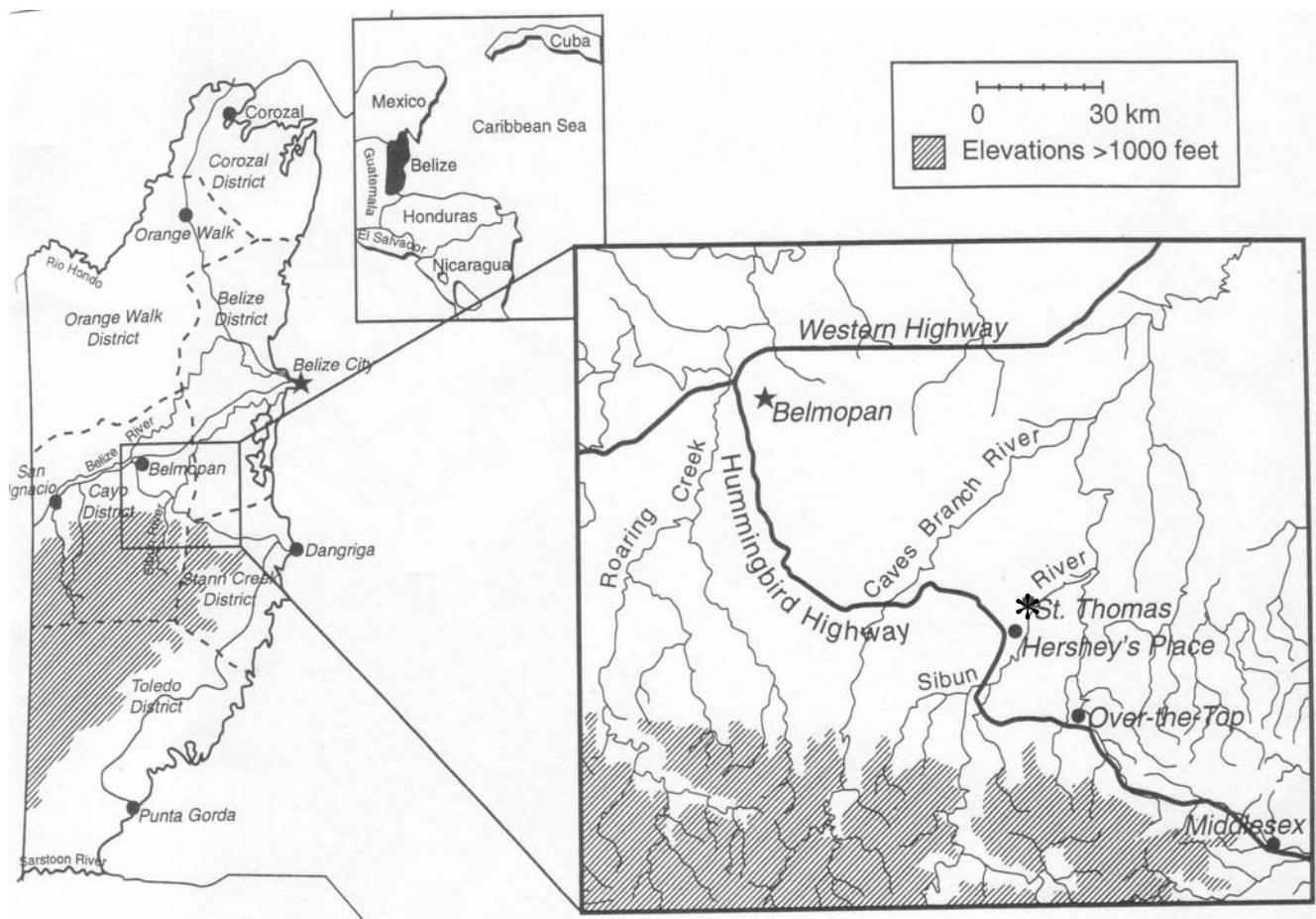


Figure 1. Map showing the location (*) of St. Thomas Creek in which the habitat preference study of *An. darlingi* was conducted (Roberts et al. 1996).

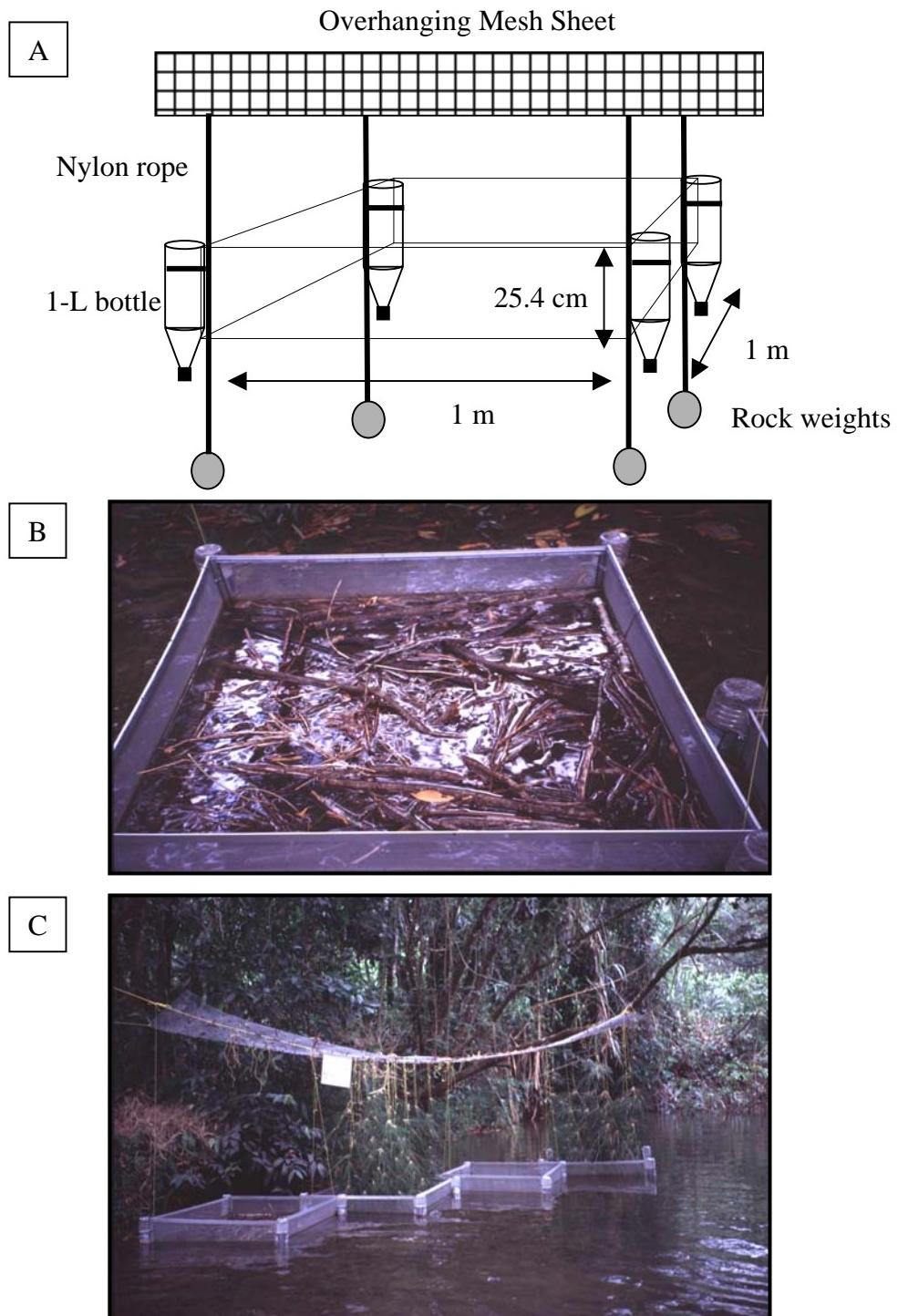


Figure 2. (A) Design of enclosure traps used in *An. darlingi* larval habitat preference study. Each trap had four screened panels and was suspended to an overhanging mesh sheet with nylon rope weighted with rocks. This allowed positional stability as well as vertical flexibility during fluctuating water levels. Trap buoyancy was created using four empty 1-L water bottles attached to each of the four corners using tie wire. (B) Picture of the detritus treatment trap and (C) one complete trap set at the St. Thomas Creek site.

Species	Larval Stage	Trap Treatment				Stage Total	Stage Contribution
		Control ^a n=12	Detritus ^b n=12	OB ^c n=12	OB + Detritus n=8		
<i>An. darlingi</i>	1	2.8	132.0	6.3	18.9	1,845	74.9%
	2	1.6	27.7	3.2	8.0	453	18.4%
	3	0.8	4.8	1.7	3.5	114	4.63%
	4	0.6	2.0	0.4	1.6	49	1.99%
	Total	69	1,997	139	256		
	Average	5.8	166.4	11.5	32.0	2,461	100%
<i>An. albimanus</i>	1	0.8	28.8	2.8	4.3	423	78.3%
	2	0.6	4.3	1.0	0	70	12.9%
	3	0.4	0.8	0.6	0	21	3.88%
	4	0.4	1.3	0.5	0	26	4.81%
	Total	27	420	59	34		
	Average	2.3	35.0	4.9	4.3	540	100%
Other ^d	1	41.4	4.8	1.2	3.0	112	61.8%
	2	0.8	0.7	0.3	0.8	27	14.9%
	3	0.4	0	0.5	1.1	20	11.0%
	4	0.2	0.7	0.3	1.1	22	12.1%
	Total	34	73	26	48		
	Average	2.8	6.1	2.2	6.0	181	100%
Trap Total		130	2,490	224	338	3,182	
Trap Average		10.8	207.5	18.7	42.3		

^aOpen water.

^bDetritus treatment consisted of dried twigs and sticks collected from the upper banks of the stream margin covering the surface water within the trap.

^cOverhanging bamboo consisted of twenty-3 ft. long sections of fresh *Guada longifolia* cut from the stream margin, inverted and tied to an overhanging mesh sheet above traps allowing leaves to touch the water surface.

^dIncludes *An. vestipennis*/*An. punctimacula*, *An. gabaldoni* and *Chagasia bathana*.

Table 1. The average number of anopheline larvae collected from floating enclosure traps during March-May 2002 sorted by species, larval instar and treatment. Each replicate (n) consisted of three sampling periods of 30 standard larval dips from each trap 5, 11 and 17 days post-setup.

Treatment	Larval Stage	Sampling Day		
		Day 5	Day 11	Day 17
Control^a		n=12	n=10	n=10
	1	10	18	6
	2	4	6	9
	3	4	0	5
	4	0	2	5
	Total	18	26	25
	Average	1.5	2.6	2.5
Detritus^b		n=12	n=10	n=10
	1	755	512	317
	2	34	79	221
	3	9	20	28
	4	5	5	14
	Total	803	616	580
	Average	66.9	61.6	58.0
OB^c		n=12	n=10	n=10
	1	18	23	35
	2	8	21	9
	3	9	6	5
	4	2	1	2
	Total	37	51	51
	Average	3.08	5.1	5.1
OB + Detritus		n=8	n=6	n=6
	1	31	86	33
	2	3	23	38
	3	4	12	12
	4	1	8	4
	Total	39	129	87
	Average	4.9	21.5	14.5
Sampling Day Total		897	822	743
Sampling Day Average		20.4	22.8	20.6

^aOpen water.

^bDetritus treatment consisted of dried twigs and sticks collected from the upper banks of the stream margin covering the trap surface.

^cOverhanging bamboo consisted of twenty-3 ft. long sections of fresh *Guada longifolia* cut from the stream margin, inverted and tied to an overhanging mesh sheet above traps allowing leaves to touch the water surface.

Table 2. The number of each *An. darlingi* larval instar collected from enclosure traps during three sampling periods sorted by treatment, species, larval instar and sampling day (i.e., 5, 11 or 17 days post-setup). Each replicate (n) represents 30 dips using a standard larval dipper from an individual trap.

Chapter 5

The use of SPOT and IKONOS multispectral satellite imagery to determine the association between land cover and bamboo growth, a potential *Anopheles darlingi* habitat producer, along river margins in Belize, Central America

ABSTRACT

Previous studies have identified several anopheline species integral to the transmission of malaria in Belize. The highly efficient vector, *Anopheles darlingi* Root, is currently considered the most important. The preferred larval habitat of *An. darlingi* has previously been described as floating patches of detritus. These patches are commonly found to be associated with overhanging spiny bamboo (*Guadua longifolia*) patches along river margins. The objectives of the following study were: 1) to use both SPOT (20 m resolution) and IKONOS (1-4 m resolution) satellite imagery to determine the association between land cover and spiny bamboo growth; and 2) to evaluate the use of high-resolution satellite imagery to detect bamboo.

Bamboo stretches were mapped in the field using hand-held global positioning system (GPS) devices within two different fresh-water river systems known to contain *An. darlingi* habitats. Buffer zones were used to quantify land cover classes adjacent to areas with and without bamboo growth. Results from field data along the Belize River indicated a total length of 26.2 km of bamboo growing along a cleared transect, comprising 60.6% (26.2/43.1 km) of the total area sampled. Within an undisturbed transect, 65.7% (24.2/36.8 km) of the total length surveyed had overhanging bamboo growth.

An unsupervised classification of the SPOT image indicated that 44.1% (2,268/5,142) of the total average pixel count encompassed within an 80 M buffer zone around the cleared transect comprised forest land cover, while 82.30% (4,237/5,113) of the pixels along the undisturbed transect were forest cover. Even though the total length of mapped bamboo was similar between transects, a significantly greater amount of forest

land cover pixels existed in the undisturbed area compared to the cleared area (chi-square=1,660; p=0.001). The overall accuracy of the unsupervised classification was 64.0%, with 84% and 88% of the forest land cover and combined cleared land cover categories (i.e., orchard, pasture/crop, bare ground/gravel) being correctly classified, respectively.

Results from sampling the Sibun River indicated a total length of 35.2 km (36.6%; 35.2/96 km) of bamboo growing along both river margins of the 48 km transect. Examination of an unsupervised classification indicated the overall average majority (56.6%; 20,049/35,451) of pixels within 4 m, 10 m and 20 m buffer zones comprised forest land cover. Orchard land cover constituted 23.1% (8,172/35,451), gravel 15.2% (5,380/35,451) and pasture only 5.22% (1,850) of the entire area. Nonparametric analyses between transects, mapped with and without bamboo growth, indicated no significant difference in pixel counts of forest, orchard, pasture or gravel land cover categories within the 4 m, 10 m or 20 m buffer zones. Similar results were seen when “cleared” (i.e., orchard, pasture and gravel) classes were combined and compared between bamboo and nonbamboo transects.

When a subset of the IKONOS image was classified using supervised training sites, 42.6% (12,233/28,733) of the total average pixel count within the 4 m, 10 m and 20 m buffer zones represented broadleaf/palm forest land cover. Sandbars and orchard categories comprised 26.4% (7,581/28,733) and 27.8% (7,980/28,733), respectively. Only 2.80% (805/28,733) of the pixels within the buffer zones were of the pasture/low grass land cover, and bare ground was the least represented category (0.47%; 134/28,733). Nonparametric analyses between transects mapped with and without

bamboo growth indicated no significant difference in pixel counts of each land cover and combined cleared classes within any of the buffer zones.

Confusion matrices for both the unsupervised and supervised classification of the IKONOS image indicated an increase in overall accuracy of pixel classification from 50.0% to 75.9% after using training sites. The classification of forest (72.4%) and pasture (87.5%) land cover had the highest accuracy rates from the unsupervised method. Orchard land cover was confused with forest (51.5%) and pasture (30.3%) categories resulting in an accuracy rate of only 18.2%. Using training sites to supervise the classification, the accuracy rate of forest land cover increased to 97.0% and pasture to 94.9%. In addition, 58.6% of the pixels within orchard training sites were correctly classified, and only 13.4% and 15.0% of the pixels, were confused with forest or pasture, respectively.

In the supervised classification of the IKONOS image, only 21.0% of the pixels within the bamboo training site were correctly classified. Pixels within training sites were most confused with broadleaf/palm forest (29.2%) but also were misclassified as orchard (14.3%) and sandbar (10.7%) land cover categories. Only 1.07% and 0.35% of the pixels were confused with pasture/low grass and bare ground, respectively. In addition, upon pan-sharpening the IKONOS image, bamboo patches did not have a consistent texture pattern, meaning that it was difficult to distinguish bamboo from other riparian vegetation growing along the riverbank.

Data presented in this study suggest that bamboo growth along riverbanks is not associated with cleared land cover adjacent to the river. In addition, high-resolution imagery does not seem to be a useful tool in distinguishing spiny bamboo from other

riparian vegetation through direct visualization; therefore it is not suggested as a cost-effective tool in detecting areas at high-risk for *An. darlingi* breeding habitats based on the presence of overhanging bamboo.

INTRODUCTION

The ability to conduct malaria risk assessments using environmental parameters is based on the basic principle that all arthropod-borne diseases are integrally related to the surrounding landscape ecology. Each vector species has a specific habitat defined by water and vegetation characteristics. These characteristics can be detected through remote sensing using satellite sensors that record spectral reflectance values at various electromagnetic wavelengths (i.e., light, heat or microwave) (Andre et al. 1995). By combining this information with spatial data, using geographical information system (GIS) tools, the researcher can then determine high-risk areas for disease transmission based upon specific parameters such as proximity to land cover types and known vector bionomics (Clarke et al. 1996). Many studies have shown the use of RS and GIS technologies to detect mosquito-breeding habitats (Hayes et al. 1985; Hay et al. 1998), predict the densities of anopheline vectors (Wood et al. 1991; Roberts and Rodriguez 1994; Pope et al. 1994; Rodriguez et al. 1996) and classify the risk of malaria transmission (Beck et al. 1994; Carter et al. 2000).

No single spatial, temporal, or spectral resolution is universally appropriate for understanding the transmission risk for a disease, given the variety of vectors, reservoirs, hosts, geographic locations, and environmental variables that might be associated with the disease (Beck et al. 2000). There are many different types of satellite sensors that can be used in remote sensing studies. Each has its own advantages and limitations. The type

of sensor a study uses will depend on the scale of area to be investigated, land cover type and budget. For example, the Landsat multispectral sensing system and the Systeme Pour l'Observation de la Terra (SPOT) with spatial resolutions of 15-30 meters/pixel and 10-20 meters/pixel, respectively, can be used for regional studies, while the IKONOS sensing system with 1-4 meters/pixel resolution is better suited for local studies. Furthermore, if a study area consists of dense vegetation or cloud cover, the RADARSAT sensing system may be necessary because it can penetrate through these conditions. However, because IKONOS high-resolution imagery has a smaller scene size (11 km^2) than Landsat (185 km^2) and SPOT (60 km^2) and is more expensive than low-resolution imagery per square kilometer, it is necessary to consider cost when deciding which sensor to use.

The public health importance of malaria in Belize has led to research focused on many anopheline species including *Anopheles darlingi* Root. This vector is presently thought to be one of the most important based on characteristics of being anthropophilic, exhibiting an endophagic feeding behavior, and natural malaria infectivity rates (Komp 1940; Kumm and Ram 1941; Achee et al. 2000; Grieco 2001; Roberts et al. 2002). In addition, this species is considered the most efficient malaria vector in the New World (Foote and Cook 1959) and, where it occurs, has been found to be the major or only vector of human malaria in South America (Forattini 1962, Deane 1986; Lourenco-de-Oliveira et al. 1989). Previous research on *An. darlingi* in central Belize has shown presence of larvae in floating mats of detritus. Mats were composed of sticks, leaves and seeds (see Chapter 4 and 6; Manguin et al. 1996). In particular, the mats were found in association with overhanging bamboo (*Guadua longifolia*) growing along riverbanks.

Remote sensing has successfully been used in Belize to predict the presence and abundance of several important endemic malaria vectors. Based upon field studies that defined the ecological determinants of *Anopheles pseudopunctipennis* larval habitats to be sunlit streams containing filamentous algae (Rejmankova et al. 1993), SPOT multispectral (i.e., 20 m resolution) satellite imagery was used to predict the presence and abundance of adults in surrounding settlements with high accuracy (Roberts et al. 1996). Adult populations of *An. albimanus* in northern villages of Belize were also accurately predicted using SPOT imagery (Rejmankova et al. 1995). These predictions were based upon distances to marshes containing both sparse macrophyte vegetation (i.e., *Eleocharis* spp.) and cyanobacteria mats, previously found to be determinants of *An. albimanus* larval habitats (Rejmankova et al. 1996, Rejmankova et al. 1993). Both types of vegetation could be detected with SPOT imagery.

The use of satellite imagery to predict *An. vestitipennis* larval habitats in Belize was not as successful (Rejmankova et al. 1998). Previous studies have shown this vector to breed primarily in marshes containing tall dense macrophyte vegetation (TDM; *Typha domingensis*) and flooded forests (Rejmankova et al. 1998; Grieco 2001). Here the difficulty occurred when the SPOT sensor could not accurately detect flooded forests and was unable to separate TDM habitat from other land cover classes including crops and pastures. While recent studies in Belize have successfully been able to predict the presence and abundance of adult *An. darlingi* at individual houses based on the distance from rivers (Roberts et al. 2002), there have been no published reports on the evaluation of remote sensing to detect areas along riverbanks at high risk for larval habitat formation using bamboo as an indicator.

Like most developing countries, Belize is experiencing an increase in landscape changes primarily due to deforestation as a result of the expansion of pastureland, citrus orchards, road construction, hydropower development and logging (Walsh et al. 1993; Land Information Center). The leading cause of deforestation in Belize is for agriculture. Types of deforestation range from large scale mechanized clearing to smaller, slash-and-burn agriculture. The cultivated sites are known as milpas. River margins are commonly associated with deforestation because of the presence of enriched soil required for citrus orchards and other crops, natural flooding events and the establishment of human settlements.

In 1996, a USAID-funded project was undertaken to determine the extent of deforestation that occurred between 1989/92 and 1994/96 on the mainland of Belize. During this time period there was a loss in forest cover of approximately 78,100 ha (White et al. 1996). The most extensive losses were reported from the Cayo and Toledo Districts where 20,090 ha and 19,035 ha, respectively, were cleared from 1989/92-1994/96. In the Cayo District, losses were concentrated around both the Belize River and Sibun River study sites for large-scale agricultural purposes. The majority of the total losses in forest cover occurred on land outside of protected areas, but a total of 8.8% (i.e., approx. 6,680 ha) of the deforestation reported by White et al. (1996) was found to have occurred in protected areas including the Sibun Forest Reserve adjacent to the Sibun River study site area. The majority of the loss within this particular reserve was the result of agricultural development along the river system consisting of citrus orchards and milpa farms. In addition, illegal land clearance within the 66-foot buffer zone (i.e., distance from high water mark) established in the Forest Reserve Act of 1996 by the Ministry of

Natural Resources has been violated in several areas of the mid-reaches of the Sibun River (personal observation).

The effect of deforestation can either increase or decrease malaria transmission depending on the resulting larval habitat formation and the bionomics of vector species within the cleared area (Walsh et al. 1993; Patz et al. 2000; Conn et al. 2002). In the Amazon, Marques (1987) pointed out that with clear-felling of the forest, erosion greatly increased and with it the silting of rivers, producing an increased tendency for rivers to overflow the banks. Such changes could produce more *An. darlingi* breeding sites when floodwaters recede along riverbanks (Rozendaal 1990). In addition, adult *An. darlingi* biting populations have been found to be scarce in the forest of Brazil (Lourenco-de-Oliveira et al. 1989). Giglioli and Charles (1954) reported the reappearance of *An. darlingi* along the Corentyne Coast of Guyana in response to ecological changes due to a newly constructed drainage-irrigation project required for a human settlement. All of these studies suggest that deforestation may favor the expansion of *An. darlingi* populations as a result of direct/indirect increases in breeding habitats. An increase in the abundance of this important vector due to land use changes could then lead to an upsurge of clinical malaria cases as reported in Brazil (Tadei et al. 1998), Guyana (Rambajan 1984) and Peru (Guarda et al. 1999).

In Belize, environmental responses to land clearing along river systems may include opportunistic growth of secondary vegetation commonly found in alluvial soils, including bamboo, that could promote the breeding of *An. darlingi* and potentially increase the prevalence of malaria. Indeed, Roberts et al. (1996) and Harbach et al. (1993) reported the collection of *An. darlingi* for the first time in almost 50 years with

previous extensive searches encountering none (Rejmankova et al. 1993; Roberts et al. 1993). These results are dependent on many factors but may include the increase in optimal *An. darlingi* habitats over time due to the expansion of bamboo growth in response to land cover change. Studies in northern Belize have reported that land cleared for agricultural purposes in close proximity to marshes has influenced the composition and density of particular vegetation species. The alteration in the type of plants within these habitats has in turn altered the composition of anopheline species (Rejmankova pers. comm.). Until the present study, no research has been performed in Belize to define the influence of land cover on the presence of spiny bamboo.

The primary objectives of the present research were to determine the ability of high-resolution satellite imagery to detect overhanging spiny bamboo and to determine the associations between bamboo growth and land cover (i.e., cleared and undisturbed) along riverbanks within central Belize. The information gathered in the current study can be used to determine the effectiveness of remote sensing to predict high-risk areas for *An. darlingi* breeding sites as a consequence of landscape changes.

MATERIALS AND METHODS

Study Sites: Transects located along both the Belize River and Sibun River systems within portions of the Cayo and Belize political districts were used to examine the association between land cover and bamboo growth (Figure 1). The study sites were chosen based upon availability of satellite imagery, land cover changes due to deforestation and known presence of *An. darlingi* larval and adult populations based on previous field data. Both rivers are part of the Central Watershed Region with a total area

of about 10, 794 km and 3,720 hectares comprising urban areas (Land Information Center).

The Belize River is part of the Belize River Watershed and extends from the Guatemalan border in the west to the Caribbean Sea in the east. Extensive deforestation has occurred within the Belize River Watershed as a result of large Mennonite settlements. The Sibun River is part of the Sibun River Watershed and has its upper reaches comprised of many small drainage streams in the Maya Mountains and lower reaches emptying into the Caribbean Sea along Belize's eastern border. Citrus production in the Sibun River Watershed constitutes the largest agricultural industry with more than 1,200 ha (1.2% of total land use) of land in some stage of cultivation (Land Information Center).

Both rivers traverse through broad-leaf forests, agricultural lands, savannah and coastal plains. The land surrounding the Belize River and Sibun River study sites has been classified as agricultural Grade 1-3 (Figure 2; Land Information Center). This designates a moderate to very high agricultural income potential, which has led to extensive deforestation primarily for citrus orchard, milpa and Mennonite crop developments (Figure 3).

Two transects were placed along the mid-reaches of the Belize River. The first corresponded to a 21.5 km cleared region (N17°12'20.4 W 89°01'41.5 / N17°13'47.0 W88°55'28.2) (Figure 4A) and the other to a 18.4 km undisturbed region (N17°22'54.1 W88°38'04.7 / N17°27'19.5 W88°36'09.1) (Figure 4B) based upon image analyses of land cover types (see below). One 48 km transect was placed along the mid-reaches of the Sibun River stretching from the Sibun bridge along the Hummingbird Highway

(N17°06'29.4 W88°39'30.9) to the village of Churchyard in the Belize District (N17°09'11.1 W88°37'26.9) (Figure 5).

Bamboo Mapping: Spiny bamboo (*Guadua longifolia*) patches growing along both riverbanks of each transect located within the Belize River were mapped during March-April 2002. Bamboo patches along the Sibun River transect were mapped in September 2002. Mapping within all transects consisted of a team of two technicians that marked the beginning and end of bamboo patches located on each side of the riverbank using Garmin III hand-held global positioning system (GPS) units (Garmin International Inc., Olathe KS). Criteria for marking a bamboo patch along the Belize River included a total length of at least 20 m and a distance from the river margin of no more than 40 m (Figure 6). Bamboo along the Sibun River was mapped providing the patch was at least 4 m long and no more than 20 m from the river margin. These criteria reduced the likelihood of biasing pixel data during classification analyses due to mixed land cover types.

Data was downloaded from the GPS units into MapSource™ 3.02 software (Garmin International Inc., Olathe KS) where line themes (i.e., bamboo and no bamboo) were created using beginning and ending data points of mapped patches from each river margin. Line themes were then transferred into ArcView® GIS 3.2 geographical information system software (ESRI Inc., Reston VA) and PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada) for further spatial analyses.

Images: Both SPOT (SPOT Image Corp., Chantilly VA) and IKONOS (Space Imaging, Inc., Thornton CO) images were used in the study. The SPOT scene with 10 M panchromatic resolution and 20 M multispectral resolution was acquired on September

10, 1998 with corner coordinates of Upper Left (UL): 89°06'30.42" W 17°33'51.83" N Lower Right (LR): 88°21'54.22" W 16°56'22.56" N. The IKONOS image with 1 m panchromatic resolution and 4 m multispectral resolution was comprised of two separate scenes. The first scene was acquired on April 29, 2002 and the second on May 29, 2002. A mosaic of the two IKONOS scenes was produced using PCI Geomatica® remote sensing software (PCI Enterprises, Ontario Canada), the corner coordinates of the final image were UL: 88°41'43.33" W 17°15'30.91" N and LR: 88°32'57.62" W 17°04'13.32" N. Both the SPOT and IKONOS images were georeferenced to a UTM projection (Zone 16 North, Row Q) with a WGS-84 datum using known ground control points collected in the field with hand-held GPS units.

Image Analyses: ArcView® GIS 3.2 geographical information system software (ESRI Inc., Reston VA) and PCI Geomatica® remote sensing software (PCI Enterprises, Ontario Canada) were used for all land cover analyses.

Land Cover and Bamboo:

SPOT Image: Two distinct transects within the Belize River (see Study Site) were defined based upon photointerpretation of land cover disturbance surrounding the river margin. One transect was placed along the Belize River where extensive vegetative clearing was apparent and the other transect in an area where no disturbance could be seen on the image. An 80 m buffer zone was then generated around each of the digitized transects. The image was classified using a 20-iteration isodata unsupervised classification algorithm to select approximately 30 land cover classes. The 30 classes were further grouped into five general categories using photointerpretation and observational field data of land cover at various sites. The five categories included: 1)

forest; 2) orchard; 3) pasture/crop; 4) bare ground/gravel; and 5) savannah/grassland. Chi-square analyses were performed to quantify the difference in pixel counts of land cover categories between transects using SPSS statistical software (version 9.0, SPSS Inc.). The total length of bamboo mapped along the riverbanks in each transect was then qualitatively compared. A confusion matrix was generated to access the accuracy of the unsupervised classification scheme in classifying land cover categories. The matrix was generated using 95 vector points placed throughout the entire image at locations that represented each land cover category with various pixel signatures

IKONOS Image: A 20-iteration, isodata unsupervised classification algorithm was used to classify the panchromatic and multispectral bands. Based on field land cover sites, photointerpretation was used to further aggregate the resulting 30 land cover classes into four general themes including: 1) forest; 2) orchard; 3) pasture; and 4) gravel.

In addition, a subset of the image was extracted (UL: 88°37'06.79" W 17°13'54.16" N and LR: 88°35'47.41" W 17°08'57.01" N) and a supervised classification was performed by a parallelepiped with maximum likelihood tiebreaker algorithm. The subset area was chosen based on the existence of several mapped bamboo patches and areas representing each land cover category of interest. Training sites used for the classification were selected from field land cover sites and included homogeneous areas of: 1) broadleaf/palm forest; 2) pasture/low grass; 3) orchards; and 4) bare ground; and 5) sandbars. Both unsupervised and supervised classifications were performed to compare analyses describing the association of bamboo growth with land cover.

Buffer zones of 4 m, 10 m and 20 m were then generated around the digitized line themes representing areas with and without spiny bamboo patches. The number of pixels

of each land cover type within the buffer zones for both the unsupervised and supervised classifications was calculated. Nonparametric analyses were performed to compare the pixel counts of individual land cover classes between bamboo and nonbamboo stretches using SPSS statistical software (version 9.0, SPSS Inc.). Aggregated land cover categories for both classifications defined as “cleared” (i.e., orchard, pasture, bare ground, gravel, sandbar) were then combined and analyses repeated for each buffer zone.

Confusion matrices were generated using remote sensing software to compare the accuracies of the unsupervised and supervised classification schemes in classifying land cover categories. The confusion matrix for the unsupervised classification was generated using 89 vector points placed throughout the entire image at locations that represented each land cover category with various pixel signatures. The confusion matrix for the supervised classification was generated using the classification of pixels encompassed within individual land cover category training sites.

Bamboo Detection: Two methods were used to determine the accuracy of IKONOS high-resolution imagery in detecting a unique bamboo spectral reflectance signature along the Sibun River. First, a parallel piped with a maximum likelihood tiebreaker supervised classification algorithm was performed using an extracted subset (see Land Cover and Bamboo). Training sites for bamboo classification were collected using field reference data within the Sibun River study site. A confusion matrix and Bhattacharrya Distance separability measure were then generated to determine the classification accuracy of bamboo compared to other land cover categories.

The second method comprised fusing the multispectral bands with the panchromatic band (i.e., pan-sharpening) and using photointerpretation of the resulting

image to access the ability of visualizing bamboo patches. Random points along the Sibun River were examined and qualitatively defined as either having bamboo or no bamboo through photointerpretation. Agreement with referenced field-marked bamboo transects was then qualitatively determined.

RESULTS

Bamboo and Land Cover:

Belize River (SPOT Image):

Results from field mapping along the Belize River indicated a total length of 26.2 km of bamboo growing along both riverbanks of the cleared transect, comprising 60.6% (26.2/43.1 km) of the total area sampled. An unsupervised classification of the SPOT image indicated that 44.1% (2,268/5,142) of the total average pixel count within the 80 m buffer zone comprised forest land cover while 37.1% (1,907/5,142) of the pixels represented orchard, pasture/crop and bare ground categories combined (Table 1; Figure 7A). Within the undisturbed transect, 65.7% (24.2/36.8 km) of the total length surveyed had overhanging bamboo growth. Classification results within this area indicated that 82.3% (4,237/5,113) of the pixels within the buffer zone were forest cover and only 0.25% (13,5113) represented orchard, pasture/crop and bare ground combined (Table 1; Figure 7B). The overall accuracy of the unsupervised classification was 64.0% (data not shown). The classification of forest land cover had an accuracy of 84.6% and the classification of combined cleared categories (i.e., orchard, pasture/crop, bare ground/gravel, savannah/grassland) had an accuracy of 88% (data not shown). Chi-square analysis indicated a significantly greater amount of non-forest pixels (i.e., orchard,

pasture/crop, bare ground/gravel, savannah/grassland) in the cleared transect compared to the undisturbed area ($p=0.001$).

Sibun River (IKONOS Image):

Results from sampling the Sibun River indicated a total length of 35.2 km (36.6%; 35.2/96 km) of mapped bamboo growing along both river margins of the 48 km transect. Examination of the unsupervised classification indicated the overall average majority (56.6%; 20,049/35,451) of pixels within the buffer zones comprised forest land cover (Table 2; Figure 8). Orchard land cover constituted 23.1% (8,172/35,451), gravel 15.2% (5,380/35,451) and pasture only 5.22% (1,850). Similar trends in land cover were seen upon examination of individual buffer zones (Table 2).

Nonparametric analyses between transects, mapped with and without bamboo growth, indicated no significant difference in pixel counts of forest ($z=-0.176$; $p=0.860$); orchard ($z=-1.076$; $p=0.282$); pasture ($z=-1.632$; $p=0.103$) or gravel ($z=-1.745$; $p=0.302$) land cover categories within the 4 m buffer zone. Similar results were seen within the 10 m buffer zone (forest; $z=-0.301$, $p=0.764$ / orchard; $z=-0.778$, $p=0.437$ / pasture; $z=-1.768$, $p=0.945$ / gravel; $z=-1.704$, $p=0.088$) and 20 m buffer zone (forest; $z=0.424$, $p=0.994$ / orchard; $z=-0.966$, $p=0.334$ / pasture; $z=-1.843$, $p=0.065$ / gravel; $z=-1.367$, $p=0.172$). Cleared land cover categories (i.e., orchard, pasture and gravel) were then combined and total pixel counts compared for each buffer zone. Again, no significant difference in the number of pixels representing cleared land cover were detected between bamboo and nonbamboo transects within the 4 m ($z=-1.125$; $p=0.261$), 10 m ($z=-0.938$; $p=0.348$) or 20 m ($z=-1.185$; $p=0.236$) buffer zones.

A confusion matrix generated for the unsupervised classification of the IKONOS image indicated an overall land cover classification accuracy of 50.0%. The classification of forest (72.4%) and pasture (87.5%) had the highest accuracy rates, while orchard land cover had a classification accuracy rate of only 18.2% (Table 3). The orchard land cover was most confused with forest (51.5%; 17/33) but was also misclassified with the pasture (30.3%; 10/33) category. Gravel land cover was sometimes confused with pasture (36.4%; 4/11).

When a subset of the IKONOS image was classified using supervised training sites, 42.6% (12,233/28,733) of the total average pixel count within buffer zones represented broadleaf/palm forest land cover (Table 4; Figure 9). Sandbars and orchard categories comprised 26.4% (7,581/28,733) and 27.8% (7,980/28,733), respectively. Only 2.80% (805/28,733) of the pixels within the buffer zones were of the pasture/low grass land cover, and bare ground was the least represented category (0.47%; 134/28,733). Similar trends were indicated for each buffer zone (Table 4).

Nonparametric analyses between transects mapped with and without bamboo growth indicated no significant difference in pixel counts of broadleaf/palm forest ($z=-1.460$; $p=0.144$); pasture/low grass ($z=-1.511$; $p=0.131$); orchard ($z=-1.762$; $p=0.078$); bare ground ($z=-0.804$; $p=0.421$) or sandbar ($z=-0.162$; $p=0.871$) land cover categories within the 4 m buffer zone. Similar results were seen within the 10 m (broadleaf/palm forest; $z=-1.503$, $p=0.133$ / pasture-low grass; $z=-1.982$, $p=0.059$ / orchard; $z=-1.114$, $p=0.265$ / bare ground; $z=-1.133$, $p=0.257$ / sandbar; $z=-1.157$, $p=0.247$) and 20 m buffer zones (broadleaf/palm forest; $z=-1.384$, $p=0.166$ / pasture-low grass; $z=-1.265$, $p=0.206$ / orchard; $z=-1.460$, $p=0.144$ / bare ground; $z=-0.487$, $p=0.626$ / sandbar; $z=-1.574$,

$p=0.115$). In addition, when cleared land cover categories (i.e., pasture/low grass, orchard, bare ground and sandbar) were combined and total pixel counts compared between transects with and without bamboo, no significant differences were detected within the 4 m ($z=-1.157$; $p=0.247$), 10 m ($z=-1.265$; $p=0.206$) or 20 m ($z=-1.870$; $p=0.061$) buffer zones.

A confusion matrix generated for the supervised classification of the IKONOS image indicated an overall land cover classification accuracy of 75.9% within training sites. Bare ground, forest, and pasture/low grass land cover categories had the highest accuracy rates with 98.8%, 97.0%, and 94.9% of the pixels being correctly classified, respectively (Table 5). The orchard and sand bar land cover classes suffered from the worse classification confusion, with 58.8% and 58.6% of the pixels, respectively, within the training areas being classified correctly. Pixels within the orchard training site were misclassified as pasture/low grass (15.0%), broadleaf/palm forest (13.4%) and sandbar (11.4%). Within the sandbar training site, the majority of misclassification (33.0%) was with the broadleaf/palm forest land cover category.

Bamboo Detection (IKONOS Image):

Following a supervised classification of a subset extracted from the IKONOS image, results from a confusion matrix indicated that 27.3% of the pixels within spiny bamboo training sites were correctly classified as bamboo land cover (data not shown). Pixels within training sites were most confused with broadleaf/palm forest (38.1%), but were also misclassified as orchard (18.7%) and sandbar (13.9%) land cover categories (data not shown). Only 1.3% and 0.46% of the pixels representing bamboo were confused with pasture/low grass and bare ground, respectively.

Examination of separability measurements (i.e. 0-1= very poor separability; 1-1.9=poor; 1.9-2.0=good) indicated similar findings in that the distinction between bamboo and sandbar land cover was very poor (0.325) (data not shown). Similar results occurred between bamboo and orchard classes (0.520). However, the ability to separate bamboo from pasture (1.71) and broadleaf/palm forest (1.12) categories was better. The best separability existed between the bamboo and forest land covers (1.99).

While some spiny bamboo patches could be manually mapped after fusing the multispectral bands of the IKONOS image with the panchromatic band (i.e., panning), many transects known to contain overhanging bamboo, referenced by field data, were not identified as bamboo through photointerpretation (data not shown). This occurred because several patches were not homogeneous for *Guadua longifolia* but were instead interspersed with other types of riparian vegetation (i.e., vines, wild cane, etc.). The different physical attributes of these plants (i.e., leaf size and shape) produced an inconsistent texture pattern on the satellite image and prevented some areas of bamboo from being properly identified.

DISCUSSION

The goal of any successful malaria control program is to reduce disease transmission in the most cost-effective manner. This can be achieved by focusing control efforts within defined high-risk areas for vector populations. Because the breeding sites of vectors are integrally associated with specific environmental determinants, the use of remote sensors as a tool to predict high-risk areas must be examined. In addition, because these habitats are influenced by landscape changes, natural or man-made, it is vital that the relationship between land cover and breeding sites be understood. Deforestation is

one important component that will change the surrounding landscape ecology and influence mosquito breeding sites (Walsh et al. 1993; Patz et al. 2000). Remote sensing and geographical information system technologies can serve as tools to understand these associations and predict changes in disease levels consequent to observed ecological changes (Andre et al. 1995; Roberts and Rodriguez 1994).

Previous investigations in Belize have characterized the vectorial roles and efficiency of some of the *Anopheles* species found throughout the country including: *An. albimanus* Weidemann, *An. pseudopunctipennis* Theobald, *An. vestitipennis* Dyar & Knab and *An. darlingi*. These species have been shown to be competent vectors in the transmission of malaria in the Americas (Loyola et al. 1991; Padilla et al. 1992; Ramsey et al. 1994; Lourenco-de-Oliveira 1989; Klein et al. 1991) and specifically in Belize (Roberts et al. 1993; Achee et al. 2000; Grieco et al. 2000); therefore, the development and evaluation of remote sensing tools to predict high-risk areas for individual vector breeding sites is an important component in the Belize malaria control program.

The environmental determinants of the target species in the present study, *An. darlingi*, have previously been identified as floating mats of detritus in shaded areas within freshwater river systems in association with overhanging spiny bamboo (Manguin et al. 1996). However until the present study, no research has examined the ability of using remote sensing to detect bamboo patches growing alongside river systems in Belize. In addition, the association between land cover adjacent to a river margin and the presence of bamboo has not been explored within this country.

The land cover categories used in the present study were kept very general (i.e., forest, orchard, pasture) because the vegetation diversity and interspersion of land cover

is high in the humid tropics, and spectral reflectance characteristics of mixed vegetation are often not distinct, causing problems in digital classification (Roy et al. 1991; Sader et al. 1991). This approach was acceptable given the goal of the research was to determine the association between “cleared” and “undisturbed” land cover categories with bamboo growth. Although congruent milpa farming is an important contributor to land cover modification, a distinct land cover class for this type of agriculture was not used in the present study. This was due to the inability of detecting milpa farms along the Belize River using the SPOT 20 m resolution image, and only a few small, individual milpas seen within the Sibun River study transect using the IKONOS 1 m resolution image. Future studies could incorporate additional land cover classes with the use of more sophisticated classification algorithms and software but this requires a higher level of training.

Data from unsupervised classification of sites along both the Belize River, using SPOT satellite imagery, and Sibun River, using high-resolution IKONOS imagery, indicate that bamboo growth along riverbanks is not associated with cleared land cover. First, even though there existed a significantly greater percentage of cleared land cover classes in the disturbed transect of the Belize River, the total length of bamboo was similar, if not slightly less, than the length of mapped bamboo in the undisturbed transect. In addition, no difference in the percentage of cleared land cover classes existed between transects mapped with and without bamboo along the Sibun River. These results were the same using either a 4 m, 10 m or 20 m buffer zone.

To evaluate the influence of image classification processes on data analyses, a supervised classification of the IKONOS image was also performed using training sites

within a subset of the Sibun River study site. Again, when transects with bamboo were compared to those without bamboo growth, no differences were found in any of the land cover categories using the 4 m, 10 m or 20 m buffer zones. Similar results were found when cleared land cover classes were combined.

Because most of the remote sensing technicians within Belize would be at the same level of training similar to the present researcher, it was important to determine the improvement of identifying land cover categories using different classification methods (i.e., unsupervised and supervised). This information could then be used to aid in determining the degree of image analyses required if these tools were incorporated into the malaria control program. These evaluations were interpreted with the use of confusion matrices that determine the amount of classification error for each land cover category specified. The unsupervised classification method resulted in an overall accuracy rate of 50.0%. This was much lower than the 75.9% generated using supervised land cover training sites. Other accuracy assessments for tropical land cover classifications have been reported as 70% in Costa Rica (Sader et al. 1991) and 73% for Belize (Spruce 1993). Even though the accuracy was higher using a supervised classification in the present study, both methods resulted in similar land cover/bamboo growth association conclusions.

Detailed examinations of the unsupervised classification confusion matrix indicated that the orchard class was mistaken as pasture land cover in 30% of the accuracy checks. The source of this confusion most likely lies in the fact that low grass (i.e., pasture-like) is found between rows of citrus trees within orchards. This would cause pixels within orchard areas to be classified as pasture; however, this confusion does

not pose a problem during analyses because both orchard and pasture classes were considered cleared land cover categories. The confusion between gravel and pasture can be explained in the same manner with similar outcomes.

The most detrimental confounding variable, using the unsupervised classification method, existed between the orchard and forest land cover categories. This misclassification comes from the inability to separate orange trees from other types of trees within the forest class. This is a problem because orchard establishment requires deforestation, and therefore, the orchard category was considered as a “cleared” land cover type during analyses. This error rate was improved by using a supervised classification of the image. Through the use of training sites, only 13.4% of the pixels within the orchard training site were misclassified as broadleaf/palm forest compared to the 51.5% using the unsupervised method. In addition, the supervised classification method resulted in an overall accuracy rate of 58.6% for the orchard land cover class compared to 18.2% resulting without the use of training sites.

In spite of these improvements, analyses using the more sophisticated supervised classification method resulted in the same conclusion as that of the unsupervised method (i.e., bamboo is not associated with cleared land cover). However, it must be stated that there are several unsupervised and supervised classification algorithms. In addition, there are also variable parameters for unsupervised (i.e., iterations, number of classes, etc.) and supervised (i.e., defining training sites) classification schemes. The classification schemes used in the present study represent only two of several possible methods but were chosen based on the relative simplicity of the objective of the study (i.e., spatial relationships with cleared and undisturbed land cover).

Because land cover did not seem to be a good indicator for the presence of bamboo patches along riverbanks, it was important to determine if high-resolution imagery could detect bamboo directly. Examination of a confusion matrix generated using a supervised classification of the IKONOS image indicated that only 21.0% of the pixels within the bamboo training sites were correctly classified. Confusion existed between the bamboo land cover and both orchard and sandbar classes. However, confusion with these classes can be rectified by physiognomic characteristics (i.e., location and geometric patterns).

The greatest misclassification of bamboo was with pixels that represented the broadleaf/palm forest land cover. This is due to the fact that several of the mapped bamboo patches were not homogenous for spiny bamboo. Other vegetation types were interspersed throughout bamboo transects. Even though care was taken to choose several bamboo training sites in various locations in order to encompass a heterogeneous population of pixel spectral reflectance, a unique spectral reflectance could not be distinguished. This poses a problem because while palm trees have characteristic textures that can be visualized and separated from bamboo growth, several other vegetative types (i.e., vines, wild cane, tall grasses and shrubs) are harder to distinguish. In addition, because the latter vegetation grows within both riparian and forested areas, separation from bamboo cannot be performed based on location. The end result is that an area along a riverbank might be indicated as having bamboo growth when actually it does not. This would be counter-effective for targeted control measures. Even with pan-sharpening of the IKONOS image bamboo could not be separated from other types of vegetation by photointerpretation.

One contributor to the misclassifications may be initial processing of the image. Upon initial acquisition of the digital data, the IKONOS image had to be “scaled” from an 11-bit image (i.e., gray scale values 0-2,047) to an 8-bit image (i.e., gray scale values 0-255) in order to fulfill the specifications of the remote sensing software required for classification. This process comprises eliminating gray level values from each band (i.e., panchromatic, red, blue, green and near-infrared) that represent outlying ranges of the image histogram. After defining the values to clip from the histogram, the pixels remaining are redistributed with the appropriate gray scale of an 8-bit image. Scaling an image is a highly subjective process and the final classification may be influenced by the analyst’s choice of where to clip the histogram.

In conclusion, data from the present research do not support the use of satellite imagery to predict *An. darlingi* larval habitats based on the presence of bamboo along river systems. There was no association between cleared land cover categories and the presence of bamboo at both the Belize River and Sibun River study sites; therefore, remotely sensed land cover is not a valuable indicator of the location in which bamboo patches will grow. In addition, because spiny bamboo could not be accurately separated from other riparian vegetation, either by computer generated classification or through photointerpretation, it would not be cost-effective at the present time to incorporate this technology in the existing malaria control program for the purposes of identifying areas at risk for *An. darlingi* habitats based on bamboo. Even though more advanced image analyses might provide more insight into the issues examined in the present study and should not be dismissed, the cost of training and/or hiring qualified personnel to conduct such analyses would have to be weighed against the benefits.

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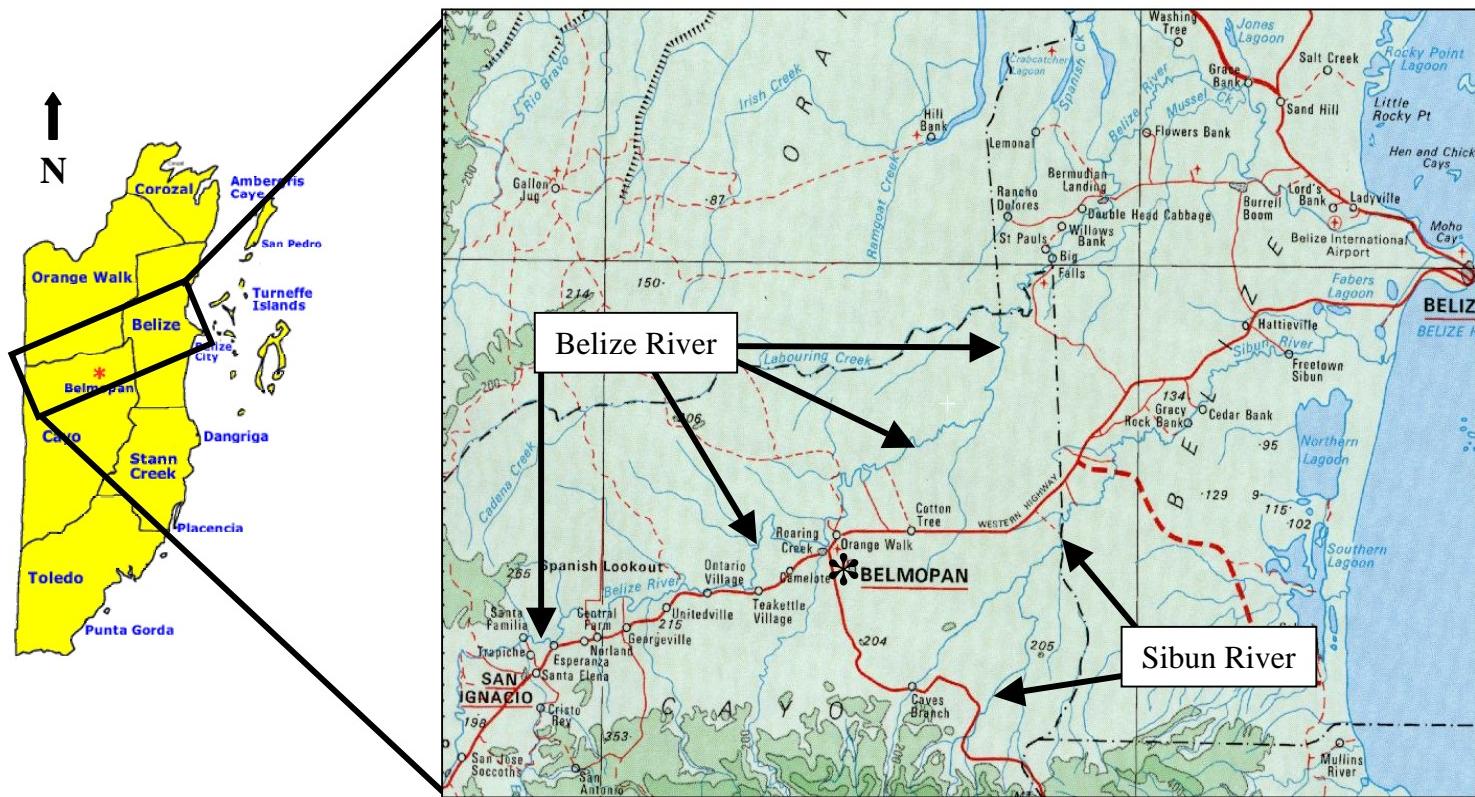


Figure 1. Bamboo growth was mapped in transects located along both the Belize River and Sibun River within the Cayo and Belize Districts.

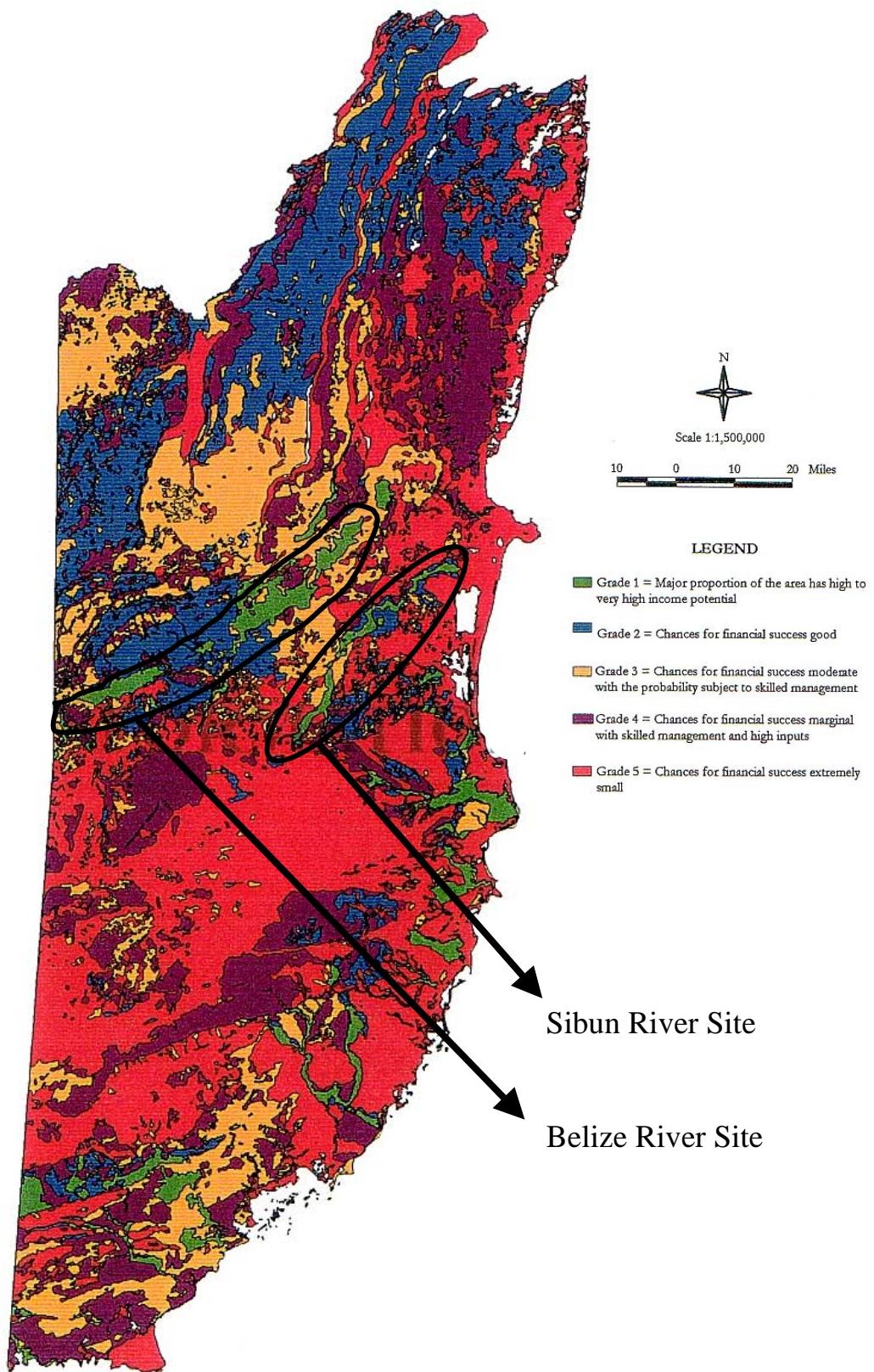


Figure 2. Agricultural grades for land use along the Belize River and Sibun River study sites in Belize, Central America (Land Information Center).

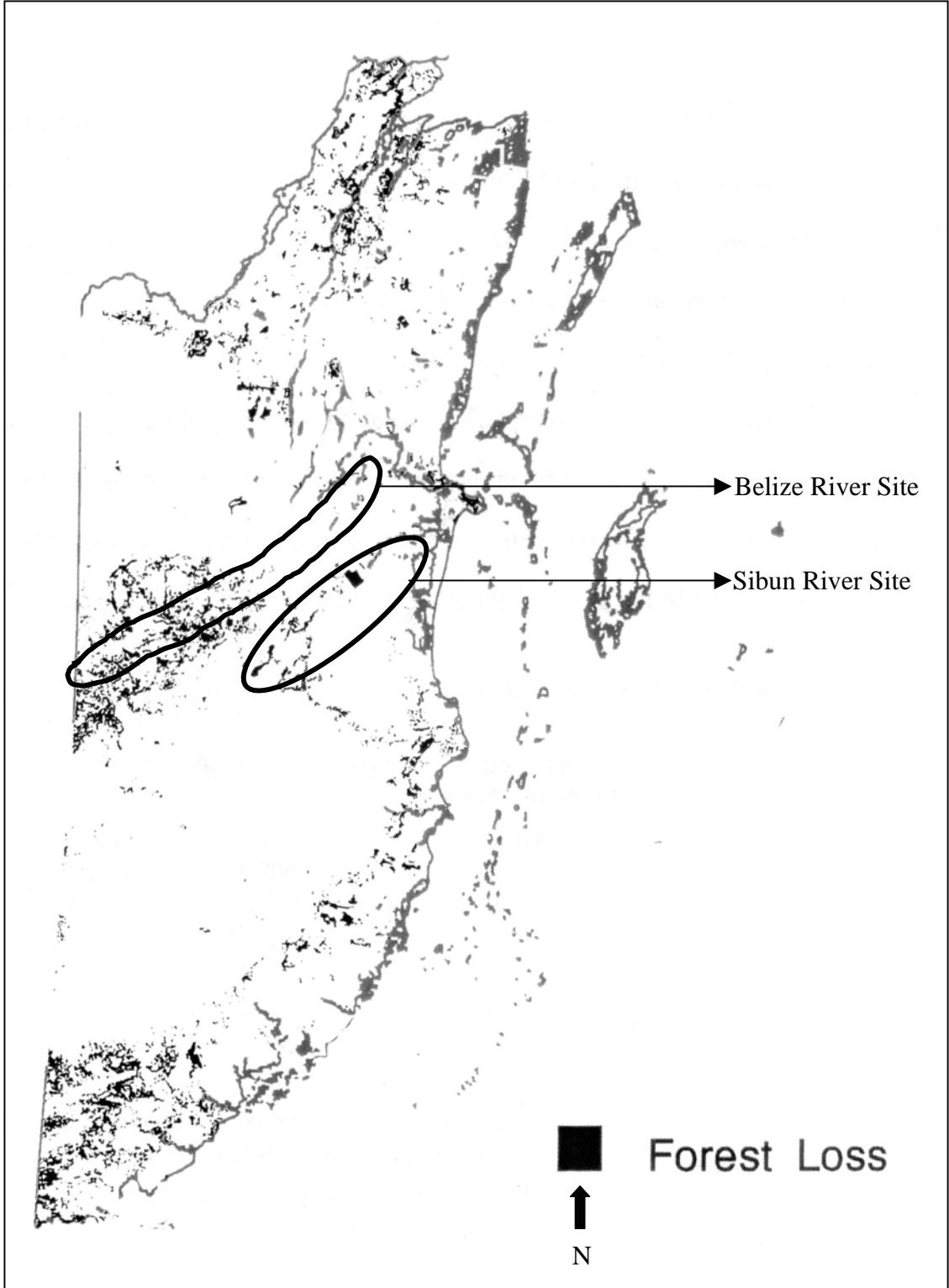


Figure 3. Location of forest and associated woodland cover losses on mainland Belize from 1989/92 to 1994 (Land Information Center).

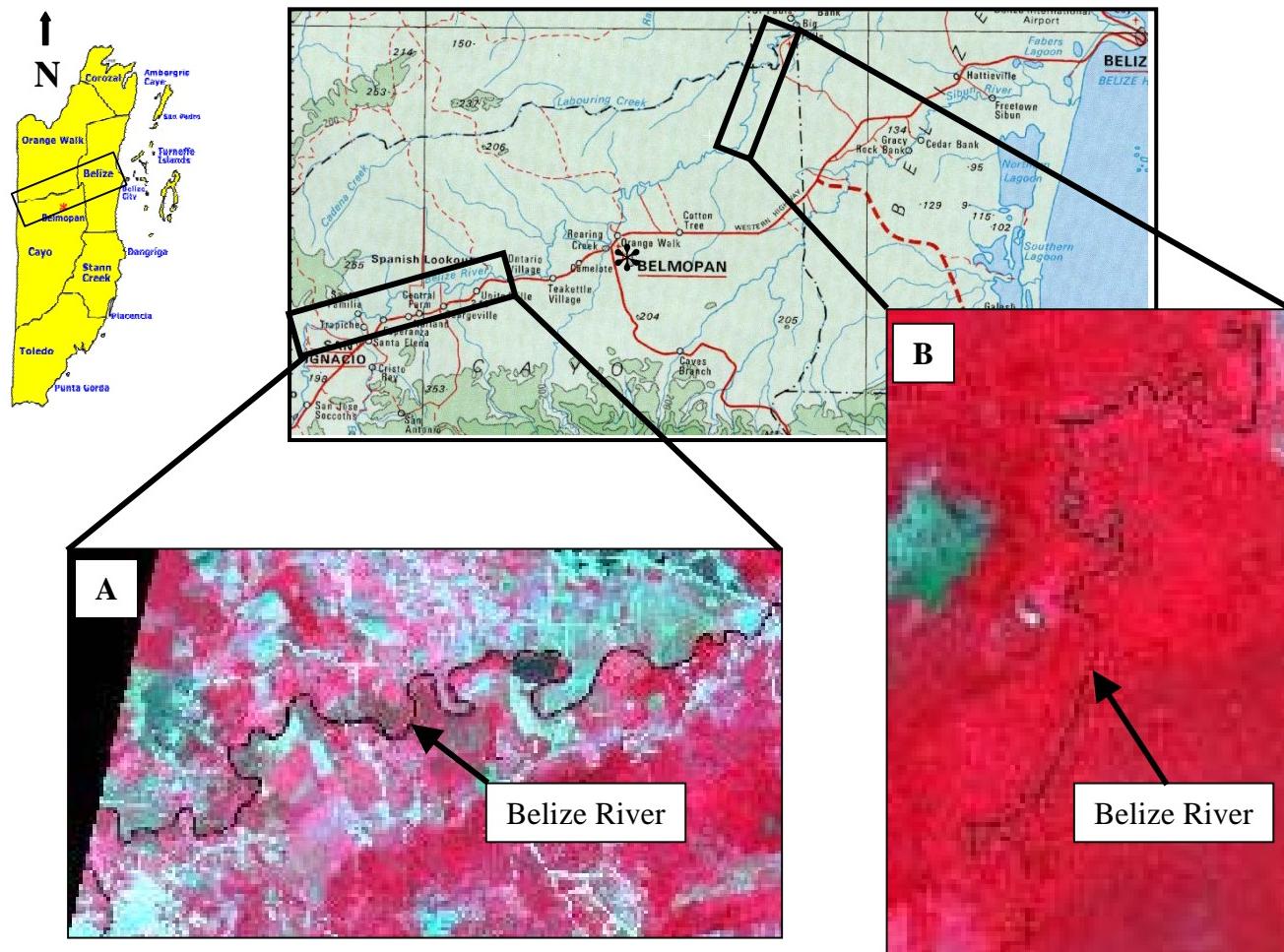


Figure 4. Sections of the Belize River designated as either (A) “cleared” or (B) “undisturbed” based upon photo-interpretation of the land cover adjacent to both riverbanks detected with SPOT 1998 multispectral satellite imagery (false color shown here). Red=non-orchard vegetation; Blue/White=bare ground, roads, pasture/crop, and orchards; Black=water.

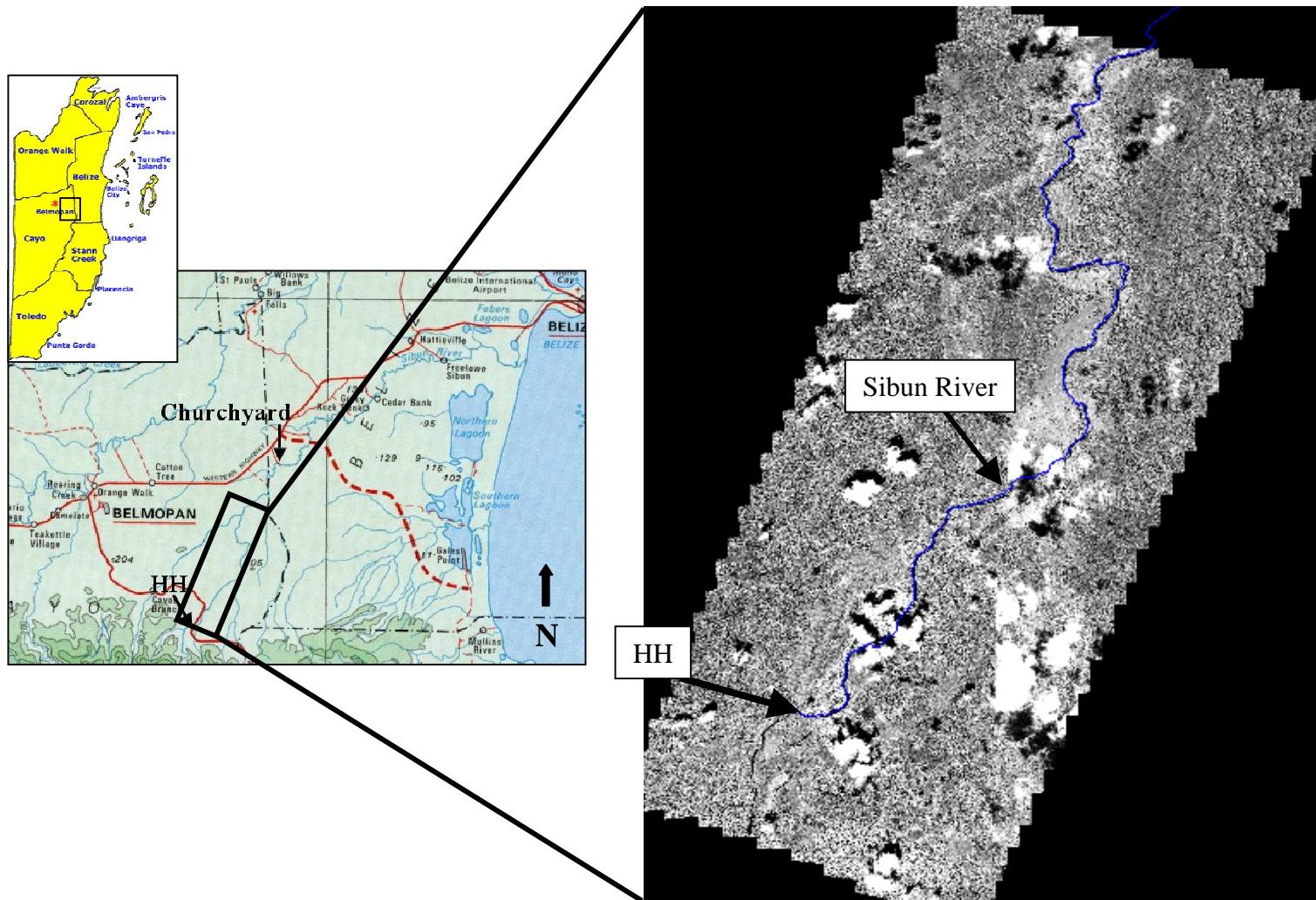


Figure 5. Bamboo patches were mapped along both margins of a 48-km transect within the Sibun River starting at the Hummingbird Highway (HH) and ending at the village of Churchyard. IKONOS 2002 high-resolution satellite imagery (the panchromatic band is shown here) was used to determine the association between land cover and overhanging bamboo.



Figure 6. An example of a patch of spiny bamboo (*Guadua longifolia*) that was mapped along the river margin of the Sibun River. Bamboo that overhangs into the surface water has previously been associated with *An. darlingi* larval breeding sites (Manguin et al. 1996).

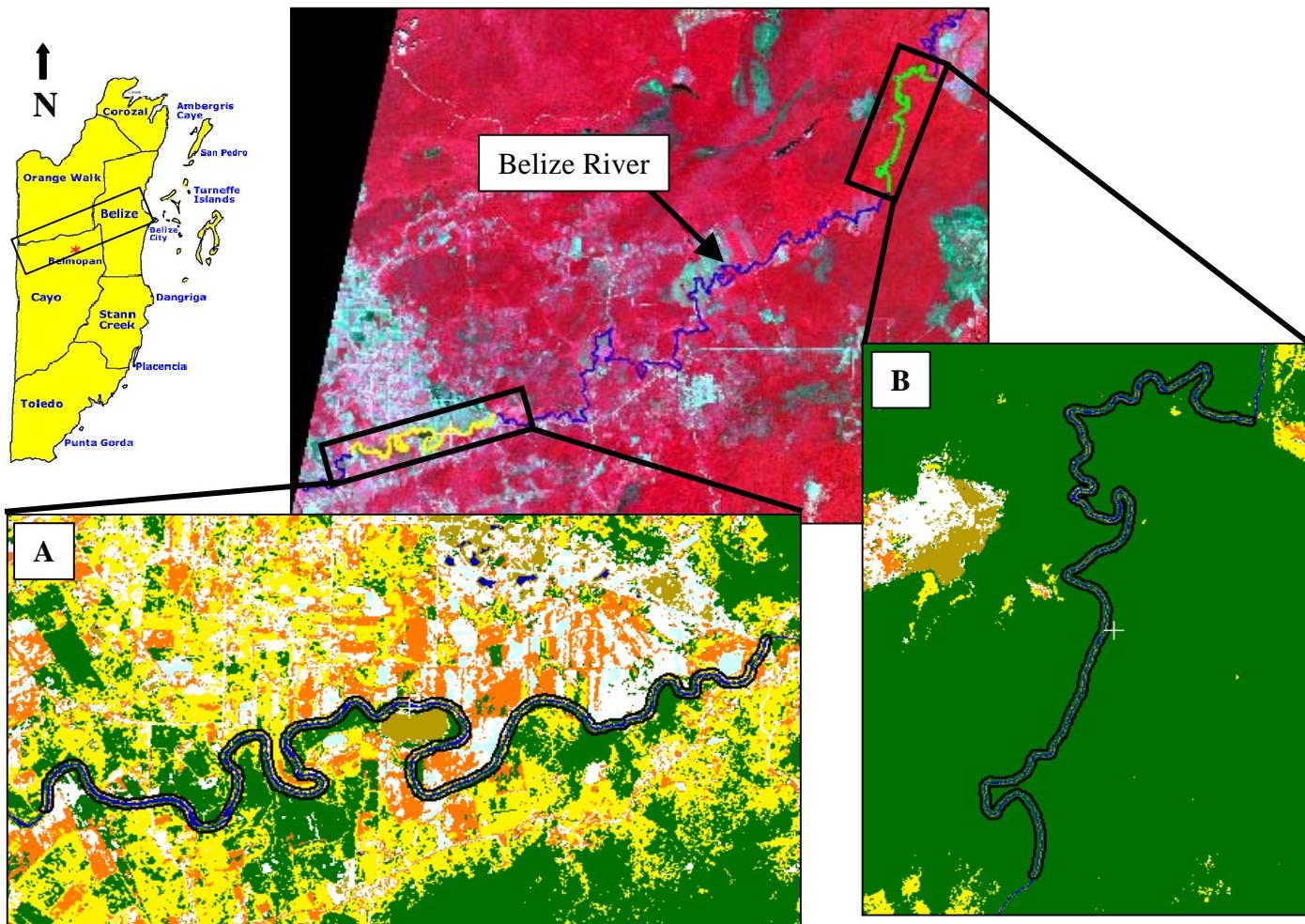


Figure 7. Results of an unsupervised classification of a 1998 SPOT scene for both the (A) cleared and (B) undisturbed Belize River transects surveyed to determine the influence of land cover on the growth of overhanging bamboo. Pixels within an 80 m buffer zone surrounding the river were categorized as either forest (green), orchard (orange), pasture/crop (yellow), bare ground/gravel (white) or savannah/grassland (taupe) based on photointerpretation of the false color composite using field land cover data.

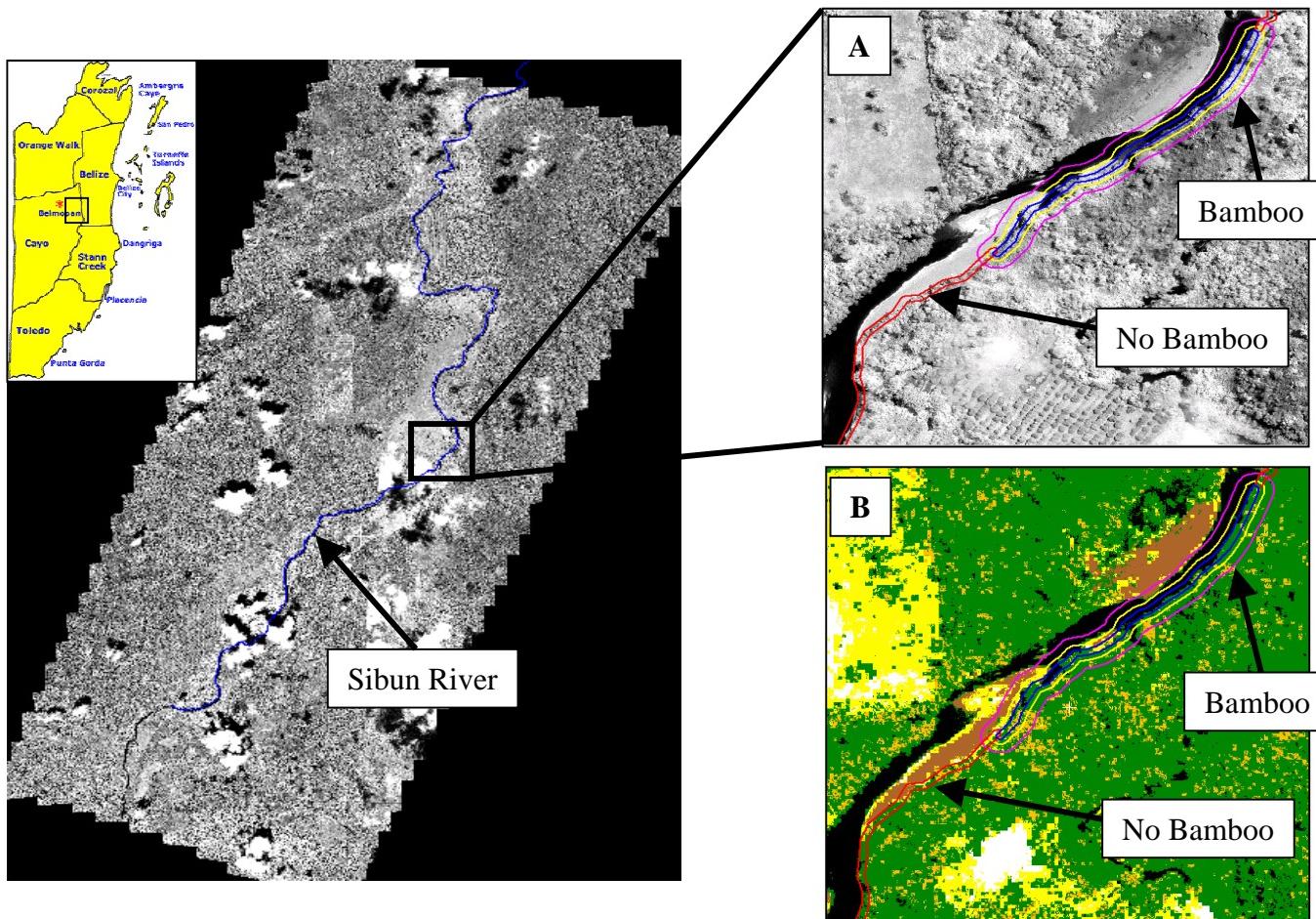


Figure 8. (A) A portion of the 2002 IKONOS image (panchromatic band) of the Sibun River study site showing the 4 m (blue), 10 m (yellow) and 20 m (pink) buffer zones generated around mapped transects with and without bamboo (only the 4 m buffer zone (red) shown). (B) An unsupervised classification of the image was used to quantify the difference in pixel counts of forest (green), orchard (orange), pasture (yellow) and gravel (brown) land cover categories between transects. Clouds are represented in white and water in black.

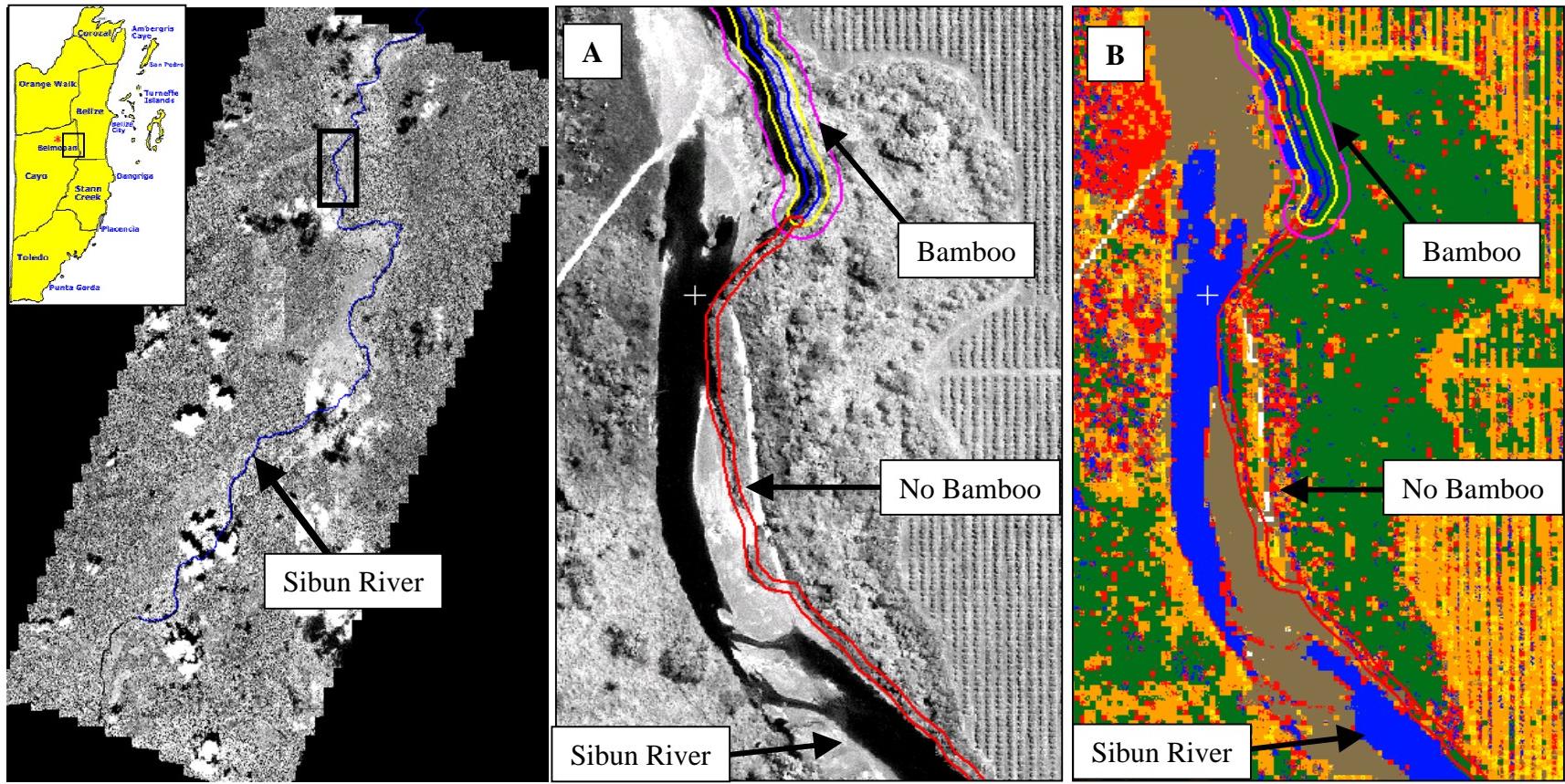


Figure 9. (A) A portion of the 2002 IKONOS image (panchromatic band) of the Sibun River study site showing the 4 m (blue), 10 m (yellow) and 20 m (pink) buffer zones generated around mapped transects with and without bamboo (only the 4 m buffer zone (red) shown). (B) A supervised classification of the image was used to quantify the difference in pixel counts of broadleaf/palm forest (green), pasture/low grass (yellow), orchard (orange), bare ground (white) and sandbar (brown) land cover categories between transects. Water is represented as black in the panchromatic band and blue in the classified figure.

Table 1. The percent land cover encompassed within an 80m buffer zone generated around a cleared and undisturbed transect within the Belize River. Bamboo was mapped along each transect to define the association between land cover and overhanging bamboo growth along river margins.

Transect	Land Cover Category ¹					Total
	Forest	Orchard	Pasture/ Crop	Bare Ground/ Gravel	Savannah/ Grassland	
Cleared	2,268	264	1,221	422	967	5,142
%	44.1%	5.13%	23.7%	8.21%	18.8%	
Undisturbed	4,237	0	8	5	863	5,113
%	82.4%	0%	0.16%	0.10%	16.8%	
Total	6,505	264	1,229	427	1,830	10,255
%	63.4%	2.5%	12.0%	4.26%	17.8%	

¹ Land cover within a SPOT 1998 scene was classified into 30 classes using a 20-iteration isodata unsupervised classification algorithm. The 30 classes were then further grouped into five general land cover categories using standard photointerpretation methods in conjunction with field land cover data.

Table 2. Results of an unsupervised classification of the 2002 IKONOS Sibun River scene showing the average percentage of individual land cover categories within a 4 m, 10 m and 20 m buffer zone generated around riverbank transects mapped with and without bamboo.

Buffer	Transect	Land Cover Category ¹				Total
		Forest	Orchard	Pasture	Gravel	
4 m	Bamboo	1,223	422	116	287	
	%	59.7%	20.6%	5.66%	14.0%	2,048
	No Bamboo	1,187	594	171	296	
	%	52.8%	26.4%	7.61%	13.2%	2,248
	Total	2,410	1,016	287	583	
	%	56.1%	23.6%	6.68%	13.6%	4,296
10 m	Bamboo	2,878	1,081	168	486	
	%	62.4%	23.4%	3.64%	10.5%	4,613
	No Bamboo	2,812	1,317	353	909	
	%	52.2%	24.4%	6.55%	16.9%	5,391
	Total	5,690	2,398	521	1,395	
	%	56.9%	24.0%	5.21%	13.9%	10,004
20 m	Bamboo	5,821	2,135	363	1,178	
	%	61.3%	22.4%	3.82%	12.4%	9,497
	No Bamboo	6,128	2,623	679	2,224	
	%	52.6%	22.5%	5.82%	19.1%	11,654
	Total	11,949	4,758	1,042	3,402	
	%	56.5%	22.5%	4.93%	16.1%	21,151
Total		20,049	8,172	1,850	5,380	
%		56.6%	23.1%	5.22%	15.2%	35,451

¹ Land cover was determined by a 20-iteration 30-class isodata unsupervised classification then further grouped into four general land cover categories using photointerpretation in conjunction with field land cover data.

Table 3. Confusion matrix of land cover determined by field sites along the Sibun River and that determined through an unsupervised classification of an IKONOS 2002 image. Reference points (89) were placed throughout the entire image at locations that represented each land cover category with various pixel signatures. Each value represents the number of counts for a particular pair of classes.

Classified Data	Field Land Cover Sites ¹				Total Counts
	Forest	Orchard	Pasture	Gravel	
Forest	21	17	1	0	39
Orchard	1	6	0	0	7
Pasture	4	10	14	4	32
Gravel	3	0	1	7	11
Total	29	33	16	11	89
% Agreement	72.4%	18.2%	87.5%	63.6%	

¹ Land cover was determined by a 20-iteration 30-class isodata unsupervised classification. The 30 classes were then further grouped into four general land cover categories using photointerpretation in conjunction with field land cover data.

Table 4. Results of a supervised classification of the 2002 IKONOS Sibun River scene showing the average percentage of individual land cover categories encompassed within either a 4 m, 10 m or 20 m buffer zone generated around transects mapped with and without bamboo growth.

Buffer	Transect	Land Cover Category ¹					Total
		Broadleaf/ Palm Forest	Pasture/ Low Grass	Orchard	Bare Ground	Sandbar	
4 m	Bamboo	843	37	436	8	312	
	%	51.5%	2.26%	26.7%	0.49%	19.1%	1,636
	NoBamboo	532	81	665	8	415	
	%	31.2%	4.76%	39.1%	0.47%	24.4%	1,701
	Total	1,375	118	1,101	16	727	
	%	41.2%	3.54%	33.0%	0.48%	21.8%	3,337
10 m	Bamboo	2,028	73	972	16	728	
	%	53.1%	1.91%	25.5%	0.42%	19.1%	3,817
	NoBamboo	1,355	158	1,365	22	1,303	
	%	32.2%	3.76%	32.5%	0.52%	31.0%	4,203
	Total	3,383	231	2,337	38	2,031	
	%	42.2%	2.88%	29.1%	0.47%	25.3%	8,020
20 m	Bamboo	4,348	143	1,872	31	1,608	
	%	54.3%	1.79%	23.4%	0.39%	20.1%	8,002
	NoBamboo	3,127	313	2,670	49	3,215	
	%	33.3%	3.34%	28.5%	0.52%	34.2%	9,374
	Total	7,475	456	4,542	80	4,823	
	%	43.0%	2.62%	26.1%	0.46%	27.8%	17,376
Total		12,233	805	7,980	134	7,581	
%		42.6%	2.80%	27.8%	0.47%	26.4%	28,733

¹ Land cover was determined by a supervised parallelepiped classification algorithm with maximum likelihood tiebreaker. Training sites for each land cover category were based on field sites.

Table 5. Confusion matrix of land cover within individual training sites determined by field land cover data and that determined by a supervised classification of an IKONOS 2002 image subset along the Sibun River.

Classified Data	Land Cover Training Sites ¹				
	Broadleaf/Palm Forest	Pasture/Low Grass	Orchard	Bare Ground	Sandbar
Broadleaf/Palm Forest	97.0%	0.05%	13.4%	0.26%	33.0%
Pasture/Low Grass	0%	94.9%	15.0%	0.05%	0.31%
Orchard	2.28%	4.18%	58.6%	0.42%	7.68%
Bare Ground	0%	0.11%	1.52%	98.8%	0.18%
Sandbar	0.68%	0.65%	11.4%	0.47%	58.8%
Total Pixels	876,216	354,883	872,463	285,267	7,358
Total %	100%	100%	100%	100%	100%

¹ Land cover was determined by a supervised parallelepiped classification algorithm with maximum likelihood tiebreaker. Training sites for each land cover category were based on field land cove data.

Chapter 6

**The use of remote sensing and GIS to define the landscape features associated with
Anopheles darlingi larval habitat presence in a fresh-water river system in
Belize, Central America**

ABSTRACT

The malaria vector *Anopheles darlingi* Root is a riverine species that breeds in floating patches of detritus. These patches form in areas along rivers where the flow is impeded and consist of various materials including sticks, leaves and seeds. A systematic survey was conducted along a 48-km length of the Sibun River in Belize to define landscape features (i.e., riverbank vegetation, house locations and water characteristics) associated with positive breeding habitats that can be used to predict high-risk areas.

All detritus patches of at least 1-m² in size were sampled, accurately located using a hand-held GPS unit and the riverbank vegetation (i.e., fallen tree, overhanging vegetation, etc.) impeding flow and causing habitat lodging characterized. A total of 54 detritus patches were sampled from both sides of the river of which 63% (34/54) contained *An. darlingi* larvae. A total of 159 *An. darlingi* larvae were captured and identified. Tree components (i.e., fallen trunks, branches and root systems) were the cause of 68.5% (37/54) detritus patch lodging, followed by bamboo components (16.7%; 9/54). In addition, these two environmental features accounted for 91.7% (33/36) of all *An. darlingi* positive patches.

Using IKONOS 4-m multispectral satellite imagery, no significant associations were found between river characteristics (i.e., deep, semi-deep, shallow water, riffles and sandbars) and positive *An. darlingi* habitats using both a 10 m computer generated and a 4 m manually generated buffer zone. However, there were significantly less deep water pixels adjacent to positive habitats compared to negative detritus mats using a manually generated 4 m polygon buffer. In addition, no significant associations were found

between positive detritus mat distribution and land cover (i.e., forest, orchard, pasture, gravel/ bare ground) within a 20 m buffer surrounding the habitat.

Although results suggest river characteristics land cover can not be used as *An. darlingi* habitat predictors, statistical analyses indicate that the average house distance from negative habitats was significantly greater than the average distance from positive *An. darlingi* detritus mats. In addition, within a 1,000 m search radius from sampled habitats there was an average 1.8 homes to every positive detritus mat compared to an average 1.4 homes to every negative detritus mat.

Because *An. darlingi* breeds in rivers, the distance to rivers is a predictor for malaria risk, but results from the present research do not support the use of remote sensing tools to predict specific locations in rivers positive for *An. darlingi* breeding sites based on land cover or river features. General areas within river systems at risk for positive detritus mats could be located by using the distance from homes to adjacent waterways, but this approach to vector management is not cost-effective. In addition, because of the dynamic characteristics of the Sibun River and transitory nature of the detritus mats, larval control is not advised.

INTRODUCTION

The ultimate goal of vector research is to define parameters that can be used to predict high-risk areas in order to target control efforts and prevent disease transmission. In 1978, the World Health Organization (WHO) revised a strategy for malaria control of which a key element was the management of disease through the reduction of larval populations. This includes the ability to detect locations of breeding habitats through remote sensing techniques. The use of remotely sensed data to predict areas at high-risk

for vector populations is based on the relationship between specific environmental variables (i.e., emergent vegetation, precipitation and surface water) and individual vector species (Andre et al. 1995). By combining field attribute data with satellite images using a geographical information system (GIS), spatial analyses can be performed and high-risk locations displayed (Clarke et al. 1996). The result is a functional tool that can be used by malaria control programs to target vector management efforts and potentially reduce disease transmission.

These technologies have been used in many vector disease studies (Roger and Williams 1993; Beck et al. 1994; Washino and Wood 1994). Based on studies in Belize that have identified the environmental determinants of several important vector species (Rejmankova et al. 1993), investigators have successfully used remote sensing to predict the location of adult *Anopheles pseudopunctipennis* (Roberts et al. 1996), the abundance of adult *An. albimanus* populations (Rejmankova et al. 1995) and the presence of both *An. punctimacula* and *An. vestitipennis* larval habitats (Rejmankova et al. 1998). The abundance of adult *An. darlingi* has been evaluated based on distance from rivers (Roberts et al. 2002); however, studies on the ability of remote sensing to predict specific locations within rivers that promote *An. darlingi* breeding sites have not been explored until the present research (see Chapters 4 and 5).

Previous studies in Belize have incriminated *An. darlingi* Root as an important malaria vector. This assessment is based on the characteristics of *An. darlingi* to feed predominately on humans (Grieco 2001), exhibit endophagic biting patterns (Roberts et al. 2002; Grieco 2001) and both its natural and laboratory *Plasmodium* infectivity rates (Grieco et al. 2001; Achee et al. 2000). In addition, this species is considered the most

efficient malaria vector in the New World (Foote and Cook 1959) and, where it occurs, has been found to be the major or only vector of human malaria in South America (Forattini 1962; Lourenco-de-Oliveira et al. 1989).

Manguin et al. (1996) described characteristics of *An. darlingi* larval habitats in Belize to consist of floating mats of detritus, including sticks, leaves and seeds. These mats were located within freshwater rivers, lakes and lagoons and were found associated with overhanging bamboo and underwater root systems. The role of spiny bamboo in *An. darlingi* habitat preference has previously been explored (see Chapter 4) and results suggest that bamboo does not serve as a selection criterion but rather facilitates lodging of detritus patches by disrupting surface water flow. The new patches might already contain *An. darlingi* larvae or provide a new egg-laying site for gravid females.

Using this information a study was conducted to determine if land use, specifically deforestation, adjacent to river systems in Belize is associated with the presence of bamboo growth along the margins (see Chapter 5). Through a combination of field studies and detailed remote sensing analyses, results indicated that cleared land cover categories (i.e., pasture, orchard and bare ground) were not associated with locations of mapped bamboo and could not be used to predict high-risk areas for potential *An. darlingi* breeding sites. However, it was necessary to determine if other features of the river and/or surrounding landscape might be useful indicators for the presence of detritus mats in order to suggest management options.

In addition, much of the land adjacent to river systems in Belize has been deforested for the promotion of citrus orchards, milpa farming and pastureland as well as a result of natural flooding events (Land Information Center). A 66-foot legal buffer zone

from the high water mark of a river has been enacted in order to prevent riverbank erosion, but lack of resources has eliminated the enforcement of this law (Forest Department; pers. observ.). The planting of bamboo along stream banks has been suggested as a corrective measure for erosion (Jorgen Rahm, pers. comm.); therefore, it was extremely important to quantify the total contribution of spiny bamboo to *An. darlingi* habitat lodging in Belize.

The objective of the present research was to define those river and landscape features that contribute the most to detritus mat lodging and distribution, and therefore promote *An. darlingi* larval populations. In addition, the use of satellite imagery to detect these features was evaluated in order to provide the Ministry of Health personnel information to guide decision making processes regarding the incorporation of remote sensing and GIS technologies into the current malaria control program for the purpose of detecting high-risk areas for *An. darlingi* breeding sites.

MATERIALS AND METHODS

Study Site: A 48-km stretch along the mid-reaches of the Sibun River in Belize, Central America was chosen as the study site based on previous research indicating the presence of *An. darlingi* adults and larvae (Manguin et al. 1996). The start of the transect was located in the Cayo District at the Sibun River bridge along the Hummingbird Highway (N17°06'29.4 W88°39'30.9) and ended at the village of Churchyard in the Belize District (N17°09'11.1W88°37'26.9) (Figure 1).

The Sibun River runs in a northeasterly direction across the central part of Belize within both the Cayo and Belize political districts. The watershed can be located geographically between 16°52' by 17°29'N latitude and 88°15' by 88°49'W longitude

and is situated in an extremely diverse geological setting (Hill 1996). The Sibun River has its headwaters in the Maya Mountains with maximum elevations of 960 meters and passes through riparian forest, a karst limestone belt, coastal marsh and mangroves through the mid- to lower reaches and finally empties in the Caribbean Sea. The Maya Mountain headwaters have an average annual rainfall between 100 and 130 inches with some of the most southern basins averaging up to 160 inches. The karst mid-reaches and the plains receive an average of 70 to 100 inches of rainfall per year (National Meteorological Service). Forestry and agriculture are predominant land uses along the mid-reaches with developed citrus plantations dominating, but small milpa farms are common. There are a total of 11 established villages (est. population of 3,000) along the Sibun River Watershed, but dispersed undocumented human habitations can be found throughout the area (Sibun Watershed Association).

Habitat and Larval Survey: Two teams consisting of 2-3 technicians each floated the river transect using fiberglass canoes in September 2002. One team surveyed the left riverbank while the other team surveyed the opposite margin. All detritus patches at least 1-m² in area were sampled (Figure 2). After marking the location of the habitat with Garmin III hand-held global positioning system (GPS) units (Garmin International Inc., Olathe KS), feature attributes including size, detritus contents and vegetation feature causing detritus lodging were recorded.

Habitats were sampled for anopheline larvae using standard plastic larval dippers (BioQuip, Gardena CA). A total of 30 dips were taken from each habitat, and larvae were placed into 6-oz. plastic Whirlpak bags (BioQuip, Gardena CA). In addition, samples of other aquatic invertebrates were collected and stored in separate bags. At the end of each

day the larvae were transferred into screw-top vials containing 80% ethanol, and the vials were labeled by a unique habitat identification code. Larvae were later identified to species (Clark-Gil and Darsie 1983), and the number of each anopheline species by individual habitat was recorded. Other aquatic invertebrates were processed as described and later identified by Dr. Roy Votsberger of Hardin-Simmons University, Abilene Texas.

Data were downloaded from the GPS units into MapSource™ 3.02 software (Garmin International Inc., Olathe KS) where point themes (i.e. habitat locations) were created using data points of mapped detritus mats from each river margin. Point themes were then transferred into ArcView® GIS 3.2 geographical information system software (ESRI Inc., Reston VA) and PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada) for further spatial analyses. Logistic regression analyses were used to define environmental parameters that were associated with *An. darlingi* positive habitats using SPSS statistical software (version 9.0, SPSS Inc.)

Image: Associations between larval habitats and landscape features were evaluated using a high-resolution IKONOS (1-m panchromatic resolution and 4-m multispectral resolution) satellite image. The Sibun River study site comprised two separate scenes. The first scene was acquired on April 29, 2002 and the second on May 29, 2002. A mosaic of the two IKONOS scenes was generated using PCI Geomatica® remote sensing software (PCI Enterprises, Ontario Canada). The corner coordinates of the final image were upper left: 88°41'43.33" W 17°15'30.91" N and lower right: 88°32'57.62" W 17°04'13.32" N. Scenes were georeferenced to a UTM projection (Zone 16 North, Row

Q) with a WGS-84 datum using known ground control points collected in the field with hand-held GPS units.

Image Analyses:

Distance to Homes and Habitat Presence: A GIS point layer was generated that defined the location of houses within the Sibun River study site based on photointerpretation of the panchromatic band (1-m resolution) of the IKONOS image using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada). Afterwards, the distances between negative and positive habitats to marked homes within a search radius of 1,000 meters were calculated and compared using nonparametric analyses.

River Characteristics and Habitat Presence: A 50-iteration, 60-class isodata unsupervised classification algorithm was used to classify river characteristics within the IKONOS image using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada). Based on field river characteristic data, photointerpretation was used to further aggregate the resulting 60 land cover classes into five general river characteristic themes including: 1) deep water (>3m); 2) semi-deep water (1-3 m); 3) shallow water (<1 m); 4) riffles; and 5) sandbars.

Buffer zones of 10 m were then generated around the digitized habitat points using ArcView® GIS 3.2 geographical information system software (ESRI Inc., Reston VA). In addition, manually produced 4 m polygon buffer zones were drawn upstream from habitats using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada). The number of pixels of each river characteristic within each type of buffer zone was calculated using remote sensing software and nonparametric analyses performed to compare the pixel counts between positive and negative habitats

using SPSS statistical software (version 9.0, SPSS Inc.). Similar analyses were performed to compare water characteristics between habitats formed by fallen trees and those formed by other landscape features.

In addition, flow rates were determined in the field by recording the average time required for a 1-in. thick foam disc of 3-in. diameter to float along a 1-m transect from three trials. Rates were determined from both riverbanks and a center location from fourteen locations within the IKONOS image scene of the Sibun River. Values were used to describe flow rates at various water depths.

Land Cover and Habitat Presence: A 20-iteration, 30-class isodata unsupervised classification algorithm was used to classify land cover within the IKONOS image using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada). Based on field land cover data, photointerpretation was used to further aggregate the resulting 30 land cover classes into four general themes including: 1) forest; 2) orchard; 3) pasture; 4) bare ground/gravel.

A 20 m buffer zone was then generated around the digitized point theme representing sampled habitats using ArcView® GIS 3.2 geographical information system software (ESRI Inc., Reston VA). The number of pixels of each land cover type within the buffer zone was calculated using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada), and nonparametric analyses were performed to compare the pixel counts of individual land cover classes between positive and negative habitats using SPSS statistical software (version 9.0, SPSS Inc.). Land cover categories representing deforested areas (i.e., orchard, pasture and bare/ground gravel) were then combined as “cleared” and pixel counts compared between habitats. Similar

analyses were performed to compare land cover types between habitats formed by fallen trees and those formed by other landscape features.

Comparison of SPOT and IKONOS images: Because the IKONOS image did not encompass the entire 48-km Sibun River transect, it was desirable to determine if a larger SPOT (20-m multispectral resolution) scene could be used for analyses. The SPOT scene was acquired on September 10, 1998 and comprised corner coordinates of upper left (UL): $89^{\circ}06'30.42''$ W $17^{\circ}33'51.83''$ N and lower right (LR): $88^{\circ}21'54.22''$ W $16^{\circ}56'22.56''$ N. A subset of the SPOT image (UL: $88^{\circ}41'44.31''$ W $17^{\circ}15'35.16''$ N; LR: $88^{\circ}32'58.21''$ W $17^{\circ}04'15.33''$ N) was made to include only the Sibun River study area of the IKONOS image. An image-to-image registration, with the IKONOS image serving as the master scene, was performed by manually selecting ground control points (GCP's) from each image using PCI Geomatica® remote sensing software (PCI Enterprises, Richmond Hill, Ontario Canada).

Both images were then classified using an unsupervised isodata 20-iteration 30-class algorithm. The resulting 30 land cover classes were further aggregated into four general themes including: 1) forest; 2) orchard; 4) pasture; and 5) gravel/bare ground. PCI's MODEL program was then used to compare the individual pixel classifications between the SPOT and IKONOS images and to generate a new image showing matching and non-matching pixels. A confusion matrix was then generated from random point sampling using remote sensing software to compare land cover differences between the images.

RESULTS

Habitat and Larval Survey:

A total of 54 detritus patches were sampled from both sides of the Sibun River transect of which 66.7% (36/54) contained *An. darlingi* larvae (Table 1). The average area of positive *An. darlingi* habitats was 3.2 m, while negative habitats were slightly larger having an average area of 5.3 meters. Examination of shade characteristics indicated that 100% (18/18) of *An. darlingi* negative habitats had shade present at any time during daylight hours. However, only 77.8% (28/36) of habitats positive for the target species had shade. The composition of detritus within all sampled mats was similar for both positive and negative *An. darlingi* habitats. Wooden sticks constituted the majority of detritus (72.2% positive; 72.4% negative) followed by leaves (19.3% positive; 16.8% negative) (Table 1). Seeds, flowers, trash and foam were also found within habitats but combined only represented 8.80% and 11.3% of the detritus within positive and negative habitats, respectively.

Statistical analyses specifically for the target species, indicated no significant difference between the size ($t=1.683$; $p=0.098$) and composition of positive and negative habitats based on sticks ($t=0.457$; $p=0.650$), leaves ($t=-0.045$; $p=0.964$), seeds ($t=1.516$; $p=0.136$), flowers ($t=0.855$; $p=0.396$), trash ($t=0.118$; $p=0.907$) or foam ($t=1.023$; $p=0.311$). However, stepwise logistical regression analyses indicated that percentage seed composition was a negative predictor for *An. darlingi* larvae ($p=0.0280$; $r=-0.2028$). No other habitat attribute data collected had significant influences with the presence of larvae.

Overall, a total of 215 larvae were sampled from the detritus mats (Table 2). Of these, 74.9% (161/215) represented the *An. darlingi* species. Other anopheline species collected were *Anopheles albimanus* Wiedemann (49) and *Anopheles pseudopunctipennis* Theobald (5). Two larvae of *Chagasia bathana* were also captured during the survey. A total of three pupae were collected of which one was *An. darlingi*, and the other two were *An. albimanus* specimens. The majority (58.6%; 126/215) of all larvae collected were of the 1st and 2nd larval stage (Table 2). Higher numbers of 3rd instar (53) than 4th instar (36) larvae were sampled. Further examination of the target species indicated that equal numbers of 1st (45/161) and 2nd (45/161) stage *An. darlingi* larvae were collected from the mats. Combined these represented 56% (90/161) of the total *An. darlingi* larval population. The number of 3rd (37/161) and 4th (34/161) instar stages were also similar and represented 44% of the population sampled.

Other aquatic invertebrates collected from the patches represented insects of several different orders including: 1) Coleoptera; 2) Collembola; 3) Diptera; 4) Ephemeroptera; 5) Hemiptera; 6) Lepidoptera; 7) Odonata; and 8) Trichoptera (Table 3). Representative specimens from each order were found in habitats with and without *An. darlingi* larvae with the exception of one Lepidopteran larva within a negative breeding site.

Habitat attribute data indicated that components of trees (i.e., fallen trunks, fallen branches and root systems) were the cause of 68.5% (37/54) of the total detritus patch lodging along the transect (Table 4). Bamboo components (i.e., live overhanging and dried fallen) comprised 16.7% (9/54). Combined, these two environmental features accounted for 85.2% (46/54) of all patch formation. Other contributors to detritus patch

lodging included overhanging vegetation other than bamboo (11%; 6/54), one eddy (2.0%; 1/54) and a single stick bridging two semi-submersed rocks (2.0%; 1/54). Of those detritus mats in which *An. darlingi* larvae were collected, 75% (27/36) were lodged by fallen trees, 16.7% (6/36) by bamboo components and 8.33% (3/36) by overhanging vegetation other than bamboo (Table 4).

Distance to Homes and Habitat Presence:

A total of 30 homes were identified through photointerpretation of the IKONOS image (Table 5). Of those, a total of 17 houses fell within the specified 1,000-m search radius used for distance analyses from either positive or negative *An. darlingi* habitats. The average distance from positive detritus mats (13) to homes (8) within the search radius was 546.32 meters, while the average distance from negative debris material (9) to houses (9) within 1,000 m was 708.49 meters (Table 5). Statistical analyses indicate that the average house distance from negative habitats was significantly greater than the average distance from positive *An. darlingi* detritus mats (Student's t-test; $t=-2.063$, $p=0.047$). In addition, there was an average 1.8 homes to every positive habitat compared to an average 1.4 homes to every negative detritus mat sampled. This represents a 23.0% (1.4/1.8) reduction in the number of homes surrounding negative breeding sites compared to habitats containing *An. darlingi* larvae.

River Characteristics and Habitat Presence:

Examination of the unsupervised classification of the IKONOS image indicated 61.9% (2,646/4,270) of the pixels within the 10 m computer generated buffer zones surrounding positive *An. darlingi* habitats to comprise semi-deep water (Table 6; Figure 3). In contrast, the majority (45.5%; 966/2,121) of pixels surrounding negative detritus

mats were of the deep water category. The riffle category comprised 7.10% (306/4,270) and 20.3% (432/2,21) of the buffer zones generated around positive and negative habitats, respectively. The sandbar category contributed the least to the total pixel count, with only 0.52% (22/4,270) surrounding positive habitats and 2.54% (54/2,121) surrounding negative detritus mats. Examination of river characteristics for habitats formed by fallen tree components indicated 48.5% (1,685/3,488) of the pixels within the buffer zone represented semi-deep water (Table 6). Riffles comprised only 8.37% (292/3,488) of the pixel count, and the sandbar category was the least represented with only 60 (1.72%) pixels encompassed within the 10 m buffer zone. Similar trends were seen for habitats formed by landscape features other than fallen trees (Table 6).

Nonparametric analyses between positive and negative *An. darlingi* habitats indicated no significant difference in pixel counts of deep ($z=-1.461$; $p=0.144$); semi-deep ($z=-1.461$; $p=0.144$); shallow ($z=-1.461$; $p=0.144$), riffle ($z=-1.095$; $p=0.273$) or sandbar ($z=0.00$; $p=1.00$) river characteristic categories within the 10 m computer generated buffer zone. Analyses of pixel counts between habitats formed by fallen trees and habitats formed by other landscape features indicated the same results.

When 4 m polygon buffers were manually placed upstream from detritus mats within the IKONOS image, trends similar to those resulting from the computer generated 20 m buffer zones were apparent (Table 6; Figure 3). Semi-deep water represented the majority, 65.9% (201/305), of the total pixel count for positive *An. darlingi* habitats. The deep and shallow water categories comprised 7.87% (24/305) and 15.7% (48/305) of the pixels, respectively. Riffles represented 10.5% (32/305) of the total count and no pixels of the sandbar category were encompassed within the buffer zones. The composition

around negative detritus mats indicated that the deep water category represented the majority 40.6% (78/192) of all pixels and semi-deep water only 34.4% (66/192). The shallow water category comprised 11.4% (22/192) of the buffer zone. Riffles represented only 13.5% (26/192) of the total pixel count, and the sandbar category was not represented. For those detritus mats formed by fallen trees, the majority of pixels (56.2%; 146/260) were of the semi-deep water category, with deep and shallow water comprising 25.0% (65/260) and 12.7% (33/260) of the pixels, respectively (Table 6). Habitats formed by landscape features other than trees had 17.7% (42/237) of the pixels represent riffle, while only 6.15% (16/260) of the pixels within buffer zones upstream from habitats formed by fallen trees represented the riffle category.

Comparison of pixel counts within the 4 m polygon buffer zones between positive and negative *An. darlingi* habitats indicated no significant difference between semi-deep ($z=-1.685$; $p=0.092$), shallow ($z=-0.447$; $p=0.655$) or riffle ($z=-0.405$; $p=0.686$) categories. However, there were significantly less pixel counts of deep water adjacent to positive habitats compared to negative detritus mats ($z=-2.201$; $p=0.028$). Nonparametric analyses between habitats formed by fallen trees and those formed by other features indicated no significant difference in pixel counts of deep ($z=-0.135$; $p=0.893$); semi-deep ($z=-0.446$; $p=0.656$); shallow ($z=-0.085$; $p=0.933$) or riffle ($z=-1.363$; $p=0.173$) river characteristic categories.

Surface water flow rates were reported from 14 locations along the Sibun River study site (Table 7; Figure 4). The river width at the locations ranged from 18-58 m and averaged 34 meters. Samples were taken at both shallow (34) and semi-deep (8) sites, however, no deep water rates were determined because of the lack of a canoe. The flow

rates at shallow sites ranged from 1.70-sec/1-m to >1-min/1-m with an average of 13.17-sec/1-m. Three shallow sites had negative flow. Surface flow at semi-deep water sites ranged from 3.96-sec/1-m to >1-min/1-m with an average rate of 20.88-sec/1-m. Statistical analyses indicated no significant difference between the average flow rates between shallow and semi-deep sites (Student's t-test; $p=0.191$).

Land Cover and Habitat Presence:

Examination of the unsupervised classification of the IKONOS image indicated the overall majority (80.2%; 13,853/17,276) of pixels within the 20-m buffer zones surrounding positive detritus mats to represented orchard land cover (Table 8; Figure 5). Pasture land cover constituted 13.3% (2,297/17,276), forest 4.47% (773/17,276) and gravel/bare ground only 2.04% (353/17,276). Similar trends in land cover were seen upon examination of buffer zones generated around negative habitats. The majority of pixels surrounding detritus mats lodged by fallen trees (79.7%; 11,012/13,811) and other landscape features (80.9%; 9,913/12,249) represented the orchard land class (Table 8). Pasture represented 12.4% (1,711/13,811) and 14.3% (1,750/12,249) of the pixels within the buffer zones of habitats formed by trees or other features, respectively. Bare ground/gravel comprised the smallest pixel counts with 2.41% (333/13,811) in buffer zones around trees and 1.01% (124/12,249) in buffer zones around habitats lodged by other landscape features.

Nonparametric analyses between positive and negative habitats indicated no significant difference in pixel counts of forest ($z=-0.465$; $p=0.642$); orchard ($z=-0.088$; $p=0.930$); pasture ($z=-0.044$; $p=0.965$) or bare ground/gravel ($z=-1.070$; $p=0.285$) land cover categories within the 20 m buffer zone. Cleared land cover categories (i.e., orchard,

pasture and gravel) were then combined and total pixel counts compared. Again, no significant difference in the number of pixels representing cleared land cover was detected between detritus mats with and without *An. darlingi* larvae ($z=-0.088$; $p=0.930$).

Nonparametric analyses between detritus mats lodged by fallen trees or other landscape features indicated no significant difference in pixel counts of forest ($z=-0.549$; $p=0.608$); orchard ($z=-0.042$; $p=0.967$); pasture ($z=-0.561$; $p=0.575$) or gravel ($z=-1.562$; $p=0.118$) land cover categories. In addition, no difference in the pixel count of cleared land cover categories existed between tree habitats and non-tree habitats ($z=-0.104$; $p=0.917$).

Comparison of SPOT and IKONOS images:

Examination of a confusion matrix generated to compare the land cover between a 1998 SPOT subset and the IKONOS 2002 scene after performing an unsupervised classification method for both images indicated an overall classification agreement of 70.0% (Table 9). Land cover indicated as Forest in the IKONOS image was correctly identified as Forest class in the SPOT subset in 95% (19/20) of the counts of Forest pixels. However, only 14.3% (1/7) and 16.7% (1/6) of the pixels representing Orchard and Pasture classes, respectively, in the IKONOS image were matched in the SPOT scene. A composite image generated to illustrate land cover pixels that did not agree between the images indicates that large areas adjacent to the Sibun River had been cleared from 1998 to 2002 (Figure 6).

DISCUSSION

The potential of maps to serve as the basis for understanding the spatial dynamics of human disease was explored beginning more than a hundred years ago (Scholten and

de Lepper 1991). The ability to predict the presence of a vector allows targeted control efforts and can be performed by spatially and temporally correlating field data of a species' bionomics with observable environmental variables. This is conducted with the use of remote sensing and geographical information system technologies (Clarke et al. 1996). Several studies have shown the success of such tools in predicting high-risk areas for malaria transmission (Beck et al. 1994; Washino and Wood 1994; Carter et al. 2000).

Several anopheline species have been previously identified to play a role in malaria transmission in Belize including *Anopheles albimanus* Wiedemann; *An. vestitipennis* Dyar and Knab; *An. pseudopunctipennis* Theobald and *An. darlingi* Root (Komp 1940; Kumm and Ram 1941; Roberts et al. 1993; Bangs 1999; Grieco 2000; Grieco 2001; Achee et al. 2000). In addition, the environmental determinants of all of these vectors have been described (Rejmankova et al. 1993; Rejmankova et al. 1998; Manguin et al. 1996) and RS/GIS tools used to predict the presence of both the larval habitats and adult presence of many (Rejmankova et al. 1995; Roberts et al. 1996). However, an evaluation of RS and GIS technologies to locate sites within rivers at high-risk for *An. darlingi* larval habitat formation has not been performed until the present research (see Chapters 4 and 5).

Among other things, the success of using satellite imagery to locate foci of interest depends on the accurate identification of environmental parameters that can serve as surrogates for vector distributions. This requires field studies to survey the ecology of larval breeding sites. Manguin et al. (1996) described *An. darlingi* habitats in Belize as dominated by floating detritus within freshwater river systems in association with overhanging bamboo. Recently conducted remote sensing studies have indicated that

bamboo growth is not associated with land cover and that satellite imagery can not be used to directly identify bamboo growing along river margins (see Chapters 4 and 5). In light of these conclusions, the total contribution of bamboo to *An. darlingi* breeding sites needed to be quantified along with other landscape features that could be used to predict habitat location.

Results from the *An. darlingi* habitat survey conducted in the present study indicated that the reduction in the density of seeds within debris material was the only habitat attribute to be significantly associated with the presence of *An. darlingi* larvae. These results are similar to personal observations made during previous larval surveys of the Belize and Sibun Rivers (Roberts pers. comm.). These observations described detritus mats that contained relatively large amounts of seeds from the Bullet Tree, commonly found along river margins in Belize, to have consistently less larvae than debris material without seeds. This led to a preliminary study of the mechanism underlying this observation. *Anopheles darlingi* larvae were placed into rearing pans with and without Bullet Tree seeds and mortality rates recorded. No effect in larval survival was observed, and a leaching mechanism of toxic material from the seeds was excluded (Roberts pers. comm.). However, this does not negate the need for more detailed chemical analyses, and perhaps future studies may find elements that could serve as larval control agents.

Another possibility for the inverse relationship between the amount of seeds trapped within detritus material and the presence of *An. darlingi* larvae is that the seeds are forming physical impediments that restrict the ability of larvae to remain in contact with the air-water interface. Also, the stability of individual larvae within a debris patch may depend on the attachment to the sticks within the mat. It is possible that the shape or

surface texture of certain seeds may preclude this attachment and force downstream drifting. The surface texture of seeds may also negatively affect the behavior of gravid females and prevent oviposition. The pre-ovipositional and egg-laying behavior of *An. darlingi* in Belize has not been examined. Detailed descriptions of such behavior under laboratory settings would provide valuable insight into how different detritus material may physically affect *An. darlingi* larvae or gravid females. Again, this may lead to innovative control methods.

Although all of the detritus patches that were negative (18/18) for *An. darlingi* larvae were shaded, results indicate that 77.0% (28/36) of the positive mats were also in the shade. This results in a total of eight patches being within sunlit areas, giving a 0.85 (46/54) probability of a potential habitat to be located at sites with shade. If we use this probability to determine the expected number of mats to be in the shade, the result is a total of 30.6 and 15.3 of the positive and negative patches, respectively. The observed values deviated from expected by approximately +/- 2 habitats. This could be accounted for by random probabilities due to the small number of total patches sampled. In addition, a patch was considered negative only if no larvae were collected, which does not necessarily mean that the detritus was not suitable for oviposition. This is reflected in the lack of statistical significance of shade between positive and negative habitats.

While predation plays an important role in the abundance of a larval population within a detritus mat, the absence of larvae within negative debris mats in the present study does not seem to be a result of a greater probability for predation. Although not systematic, representative samples of predatory insects (i.e., Coleoptera, Diptera, Hemiptera) were collected from both negative and positive habitats. More importantly,

the average area of sampled negative debris patches was larger than those containing *An. darlingi* larvae. Because the area difference was not significant, results probably reflect the difficulty in collecting larvae from detritus mats that encompass a bigger area. Other reports from Belize have documented the difficulty in sampling *An. darlingi* larvae because of their ability to camouflage with the wood pieces and their ability to adhere to the upper surface of detritus when the water surface is disturbed during dipping (Manguin et al. 1996).

Exploratory analyses from the Sibun River survey indicated that overhanging vegetation, bamboo or otherwise, did lodge debris, but that tree components (i.e., fallen trunks, branches and root systems) contributed to the majority of all detritus mat lodging and positive *An. darlingi* habitats compared to other landscape features. Management of these features would include the removal of fallen trees from rivers and the trimming of overhanging vegetation that touches the surface water. Naturalistic control methods have been previously suggested to control malaria vectors (Muirhead-Thomson 1951). Within the Philippines, “stream-clearing” or the removal of riparian vegetation has been used as a larval control method for *An. flavigaster* because the vector breeds in shaded sites along riverbanks (Foley et al. 2002). Management of riparian vegetation is an attractive option because it offers potentially long-term control and is achievable with community participation and village-based resources. However, because of the transitory nature of detritus mats and the dynamic characteristics of the Sibun River, the efficacy of stream-clearing to control *An. darlingi* larval populations in Belize is not advised.

Results from the present survey in combination with other remote sensing studies (see Chapter 5) signify that the use of overhanging bamboo as an efficient indicator of

An. darlingi larval habitat distribution cannot be supported. Therefore, associations between the locations of detritus mats with river characteristics were investigated. It should be noted that previous studies (Manguin et al. 1996) found no associations between positive debris patches and either pH or conductivity; therefore, water samples were not taken during the present survey. Examination of the composition of pixels within computer-generated 10 m circular buffer zones generated around both positive and negative habitats indicated no significant correlations with water depth (i.e., deep-shallow) or the presence of riffles or sandbars. In addition, these same river characteristics could not be used to distinguish locations of habitats formed by tree components or those formed by other landscape features. However, the majority of sampled mats were lodged by fallen trees, which impeded downstream flow, so the role of these downstream water characteristics that were encompassed in the circular buffer zone to habitat suitability was considered minimal.

In order to assess this theory, a manually generated 4 m polygon buffer zone was placed upstream from the detritus mat and analyses repeated. Again, the composition of pixels representing varying river characteristics was not correlated with the presence of habitats formed by fallen trees or other landscape features. Analyses with the polygon buffers did indicate positive detritus mats had significantly less deep-water pixels upstream than negative debris patches. This is similar to findings reported by Manguin et al. (1996) that described *An. darlingi* habitats to be located at locations within the Sibun River that were at a depth of <0.2 m, although this was not a significant indicator. In addition, the hydrology patterns of the Sibun River are not stable due to flooding and gravel mining within the area. Water depth and surface flow rates can vary daily,

especially in the wet season but also during the dry season, due to precipitation levels and man-made dredging (pers. observ.). Because of this, remotely sensed data of the river system may not reflect accurate river characteristics within the field and would render the use of satellite imagery to guide larval control efforts ineffectual.

Another river characteristic that may influence the location of fallen trees and therefore positive *An. darlingi* habitats is the location of curvatures, or bends, in the system. Preliminary data exploration in the present study indicated that detritus mats lodged by fallen trees were randomly positioned throughout the Sibun River transect (data not shown) at sites with and without bends. Detailed analyses of the rate of change in the river angle surrounding these locations would give insight into the predictive capability of this parameter and should be investigated in the future.

Fortified with the knowledge that fallen trees contributed to the majority of positive *An. darlingi* detritus patches, it was important to describe the correlations between adjacent land cover and the presence of these landscape features. Deforestation along the Sibun River is common for the purposes of large-scale orchard and pastureland establishment, small-scale milpa farming as well as through natural flooding events. In addition, gravel mining for road construction is widely practiced within the study area (Sibun Watershed Association). This deforestation may lead to erosion of the riverbanks during periods of high precipitation, common in the central region of the country during the rainy season, and cause trees along the margin to uproot and fall into the river. If this was true then land cover may be an effectual indicator for the location of potential larval habitats.

Results indicated that forest, orchard, pasture or bare ground/ gravel land classes were not indicators for the presence of positive debris patches compared to negative habitats as well as those mats formed by fallen trees versus other landscape features. This was also true when the cleared categories were combined. Because the overall landscape within the study site contains several large orchards, the fact that the pixels within the 20 m buffer zone surrounding mapped habitats represent the orchard category is a result of the high probability of a detritus patch being located adjacent to orchards along river margins and does not imply the distribution of detritus patches was clustered in orchard areas. Results of the statistical analyses within the present study validate this statement.

The analyses performed in the current study were limited to the area covered by the IKONOS 2002 high-resolution satellite image. From a total of 54 detritus patches sampled along the 48-km transect, only 28 mats were visible on the IKONOS scene. The remaining 26 were either off the image (21) or under clouds (5). For this reason, a comparison between a SPOT 1998 and the IKONOS 2002 image was performed in hopes of increasing the sample size of habitats for comparisons. While the overall agreement between land cover was 70.0%, those pixels in the SPOT image that did not match the IKONOS image were in key areas of cleared land. This reflects changes in land use between the four years and decreased the confidence in using the SPOT scene for analyses. Although misclassification elements within one or both dates can often create false change classes (Woodwell et al. 1983), the general land cover categories used in the present study (i.e., forest, orchard, pasture, bare ground/ gravel) are easy to distinguish through physiognomic characteristics. Although costly, it would be fruitful for future studies to mosaic a second IKONOS image with the current scene and repeat analyses

with the entire data set in order to determine if conclusions would differ from the current research.

The only indicator of *An. darlingi* habitats determined by the present study was the distance to surrounding homes. Those debris mats not containing larvae of the target species were an average 162.17 m further away from marked homes compared to positive habitats. In addition, *An. darlingi* positive patches had 23% more homes within a 1,000 radius than negative mats. These conclusions are similar to those of other studies in Belize showing remote sensing and GIS can be used to predict malaria risk based on distance of homes to rivers. Examination of malaria prevalence in Belize during 1989-1999 found proximity to a stream (i.e., <1 km) predictive for malaria within a household (Hakre 2003). In addition, Roberts et al. (2002) found the distance from homes to rivers to be a good predictor of adult *An. darlingi* during the wet season. In a study that investigated the ability of multispectral satellite data to predict the location of adults of another vector, *An. pseudopunctipennis*, *An. darlingi* adults were also collected at all high probability sites and none were captured at houses predicted as low probability (Roberts et al. 1996). The criteria for site selection included distance of houses from waterways. These findings in combination with other data from studies in Belize (see Chapter 3) and South America (Charlwood and Alecrim 1989) continue to indicate *An. darlingi* to be anthropophilic and synanthropic.

It must be stated, however, that detritus patches in the current study were labeled negative only if no *An. darlingi* larvae were found during sampling. This does not mean that a negative patch was unsuitable for oviposition. Eggs could have been present in negative detritus mats and therefore, larvae would have been collected if repeated

samples of the debris patch were taken over time. Knowing that *An. darlingi* will fly long distances (i.e., 800 m-7 km) for a human bloodmeal (see Chapter 3; Charlwood and Alecrim 1989; Deane et al. 1948), the adult population generating from these mats would create a malaria risk to the inhabitants of those homes within the 1,000-m search radius. For these reasons, the significance of finding a difference of 162.17 m when comparing distances of positive and negative breeding sites to surrounding houses must be taken in perspective.

In conclusion, the majority of *An. darlingi* larval habitats sampled within the Sibun River were lodged by tree components including fallen trees. No relationships between these landscape features and either river characteristics (i.e., water depth, riffle and sandbar location) or land cover (i.e., forested and disturbed) was indicated. The distance from a positive detritus patch to homes within 1,000 meters was, however, significantly less than the distance from negative habitats. High-resolution satellite imagery could be used to detect homes along river systems and potentially predict general areas along river systems at risk for *An. darlingi* breeding habitats based on distances from houses to waterways; however, the cost of such images (i.e. ~\$6,000.00 US/scene) may preclude the effectiveness of such tools in malaria control programs within developing countries such as Belize. In addition, the management of detritus patches through removal of fallen trees and trimming of overhanging vegetation in targeted areas may not be feasible due to a lack of resources and dynamic nature of the Sibun River. The reduction of *An. darlingi*/human contact is best targeted through the adult vector population.

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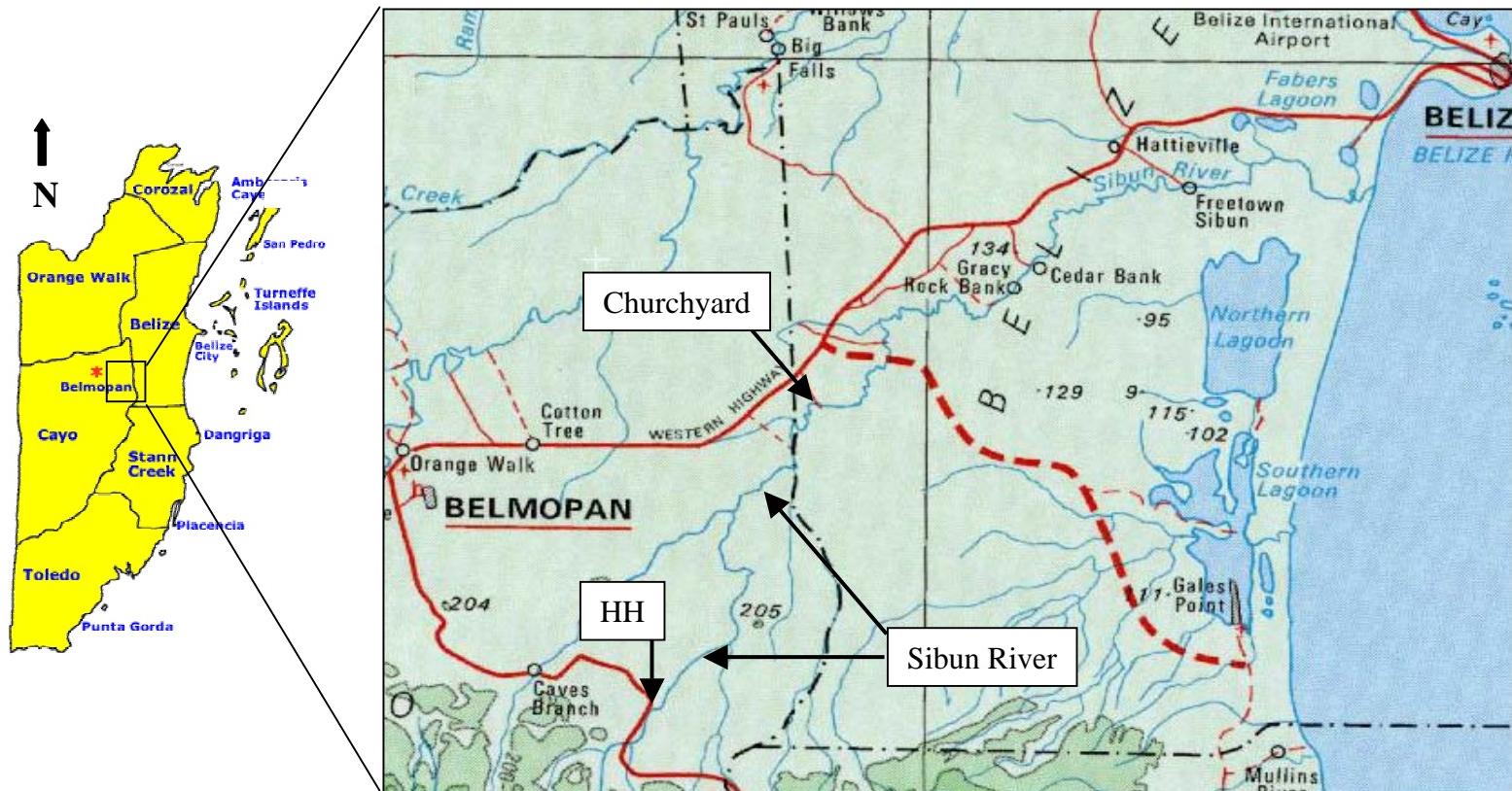


Figure 1. A survey of *An. darlingi* larval habitats was conducted along both sides of the Sibun River within a transect that started at the Hummingbird Highway (HH) and ended at the village of Churchyard. Sampling was conducted to define the associations between landscape features and the presence of breeding sites.

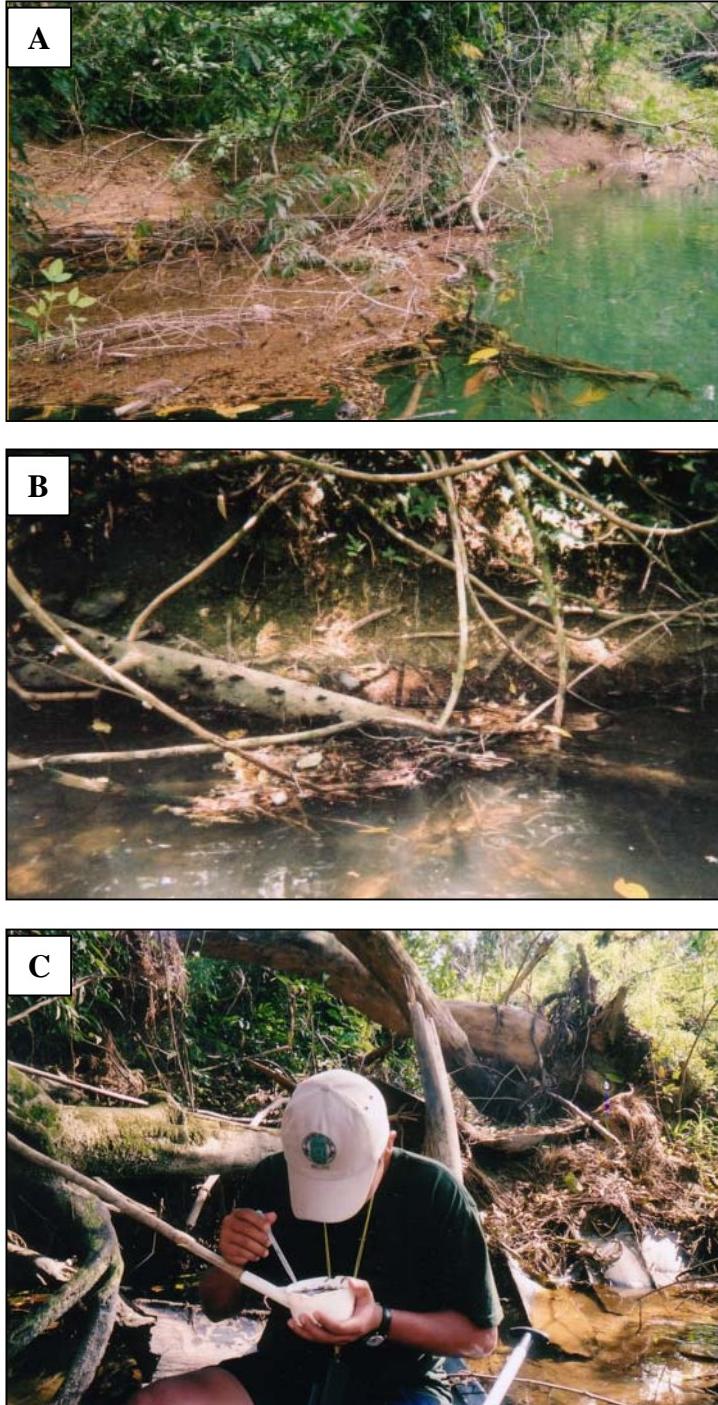


Figure 2. All detritus patches of at least 1-m² in area were sampled along the Sibun River transect for *An. darlingi* larvae. The locations of sampled habitats were marked using global positioning system units and feature attributes including size, detritus contents and vegetation feature causing detritus lodging were recorded. (A-B) Typical habitats found during the survey. (C) Habitats were sampled for anopheline larvae using standard plastic larval dippers.

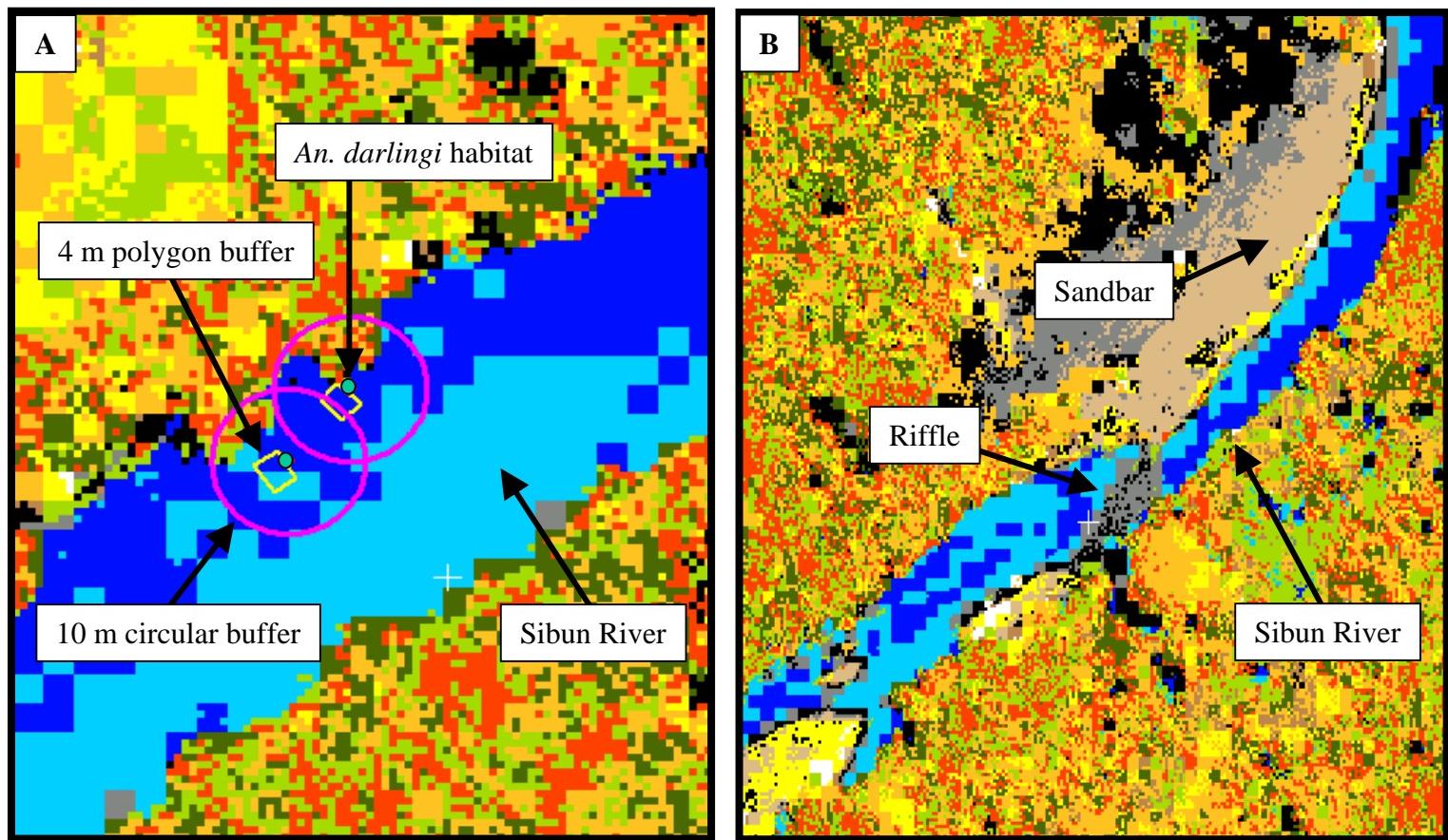


Figure 3. A portion of the unsupervised classification of an IKONOS 2002 image illustrating (A) both the 10 m computer generated circular and 4 m manually generated polygon buffer zones used to analyze the association between *An. darlingi* habitats and (B) river characteristics (i.e., water depth, riffles and sandbars). Detritus mats were sampled along a 48-km transect of the Sibun River in September of 2002. In this figure cyan represents shallow water (<1 m) and purple semi-deep water (1-3 m).

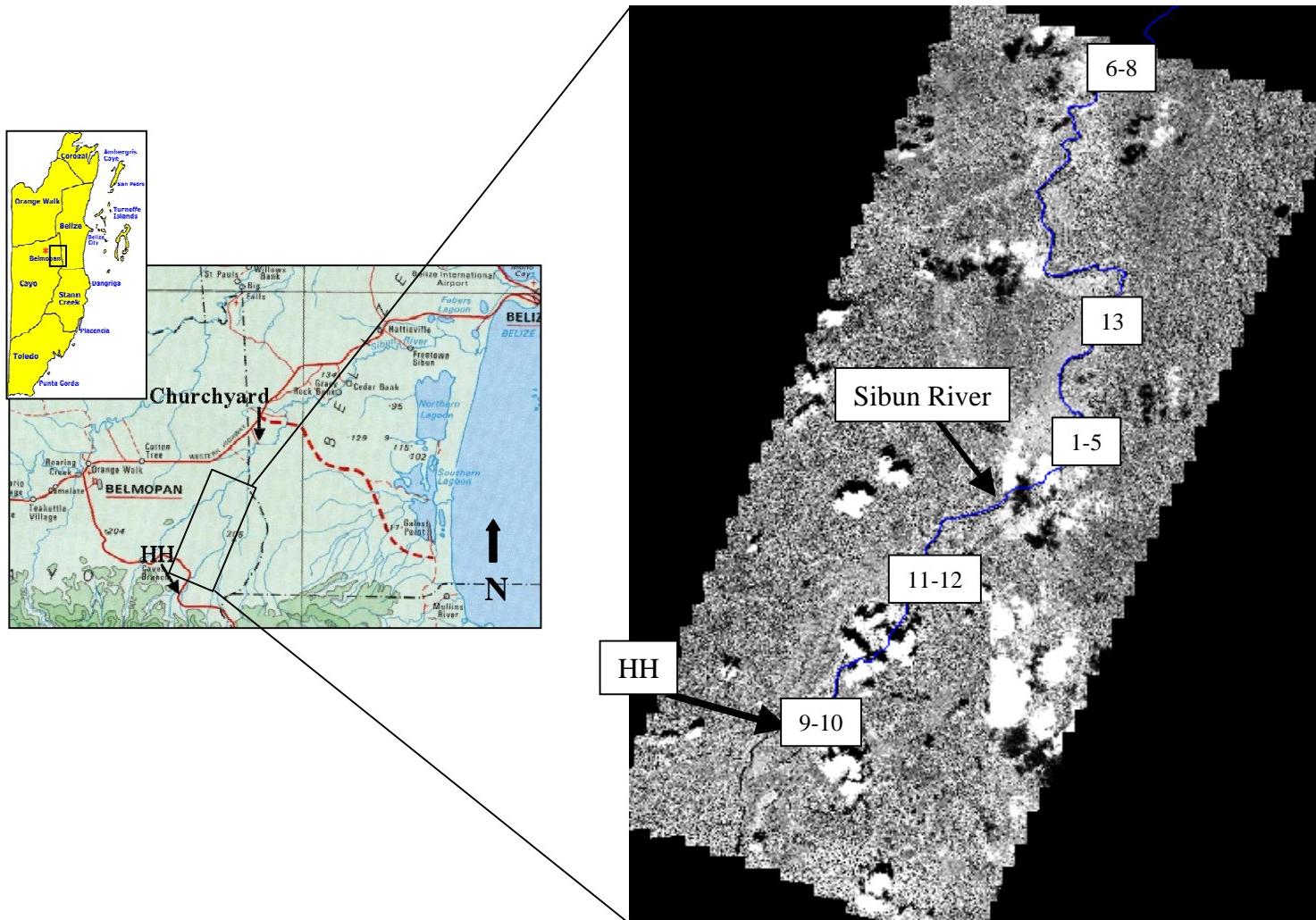


Figure 4. Location along the Sibun River study site where samples of surface water flow rates were taken in September 2003.

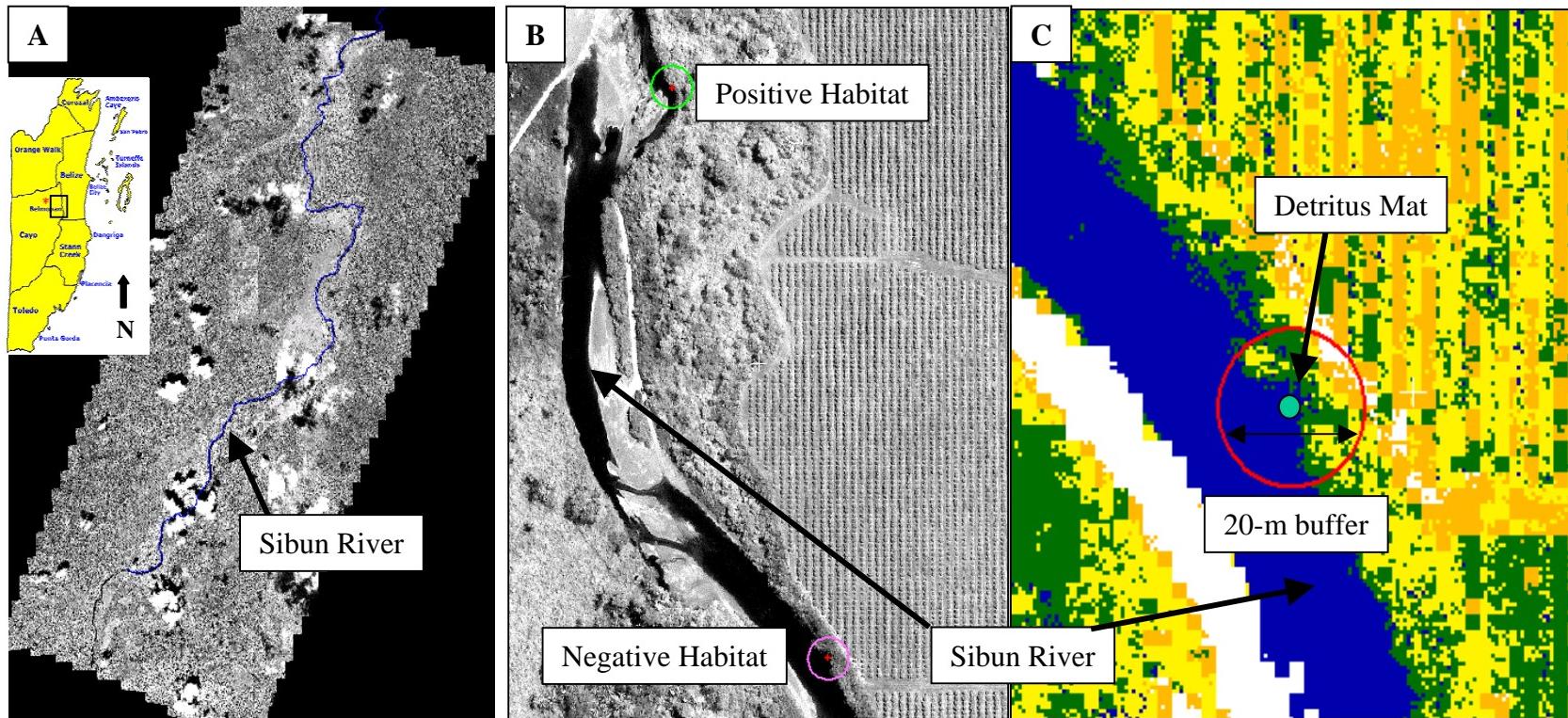


Figure 5. (A) An IKONOS satellite image (panchromatic band shown) was used to determine the association of land cover to the presence of detritus mats mapped during a survey of the Sibun River in September 2002. (B) 20-m buffer zones were generated around both positive and negative *An. darlingi* detritus habitats and (C) an unsupervised classification performed to categorize pixels into four land cover categories including: forest (green); orchard (orange); pasture (yellow) and gravel/ bare ground (white).

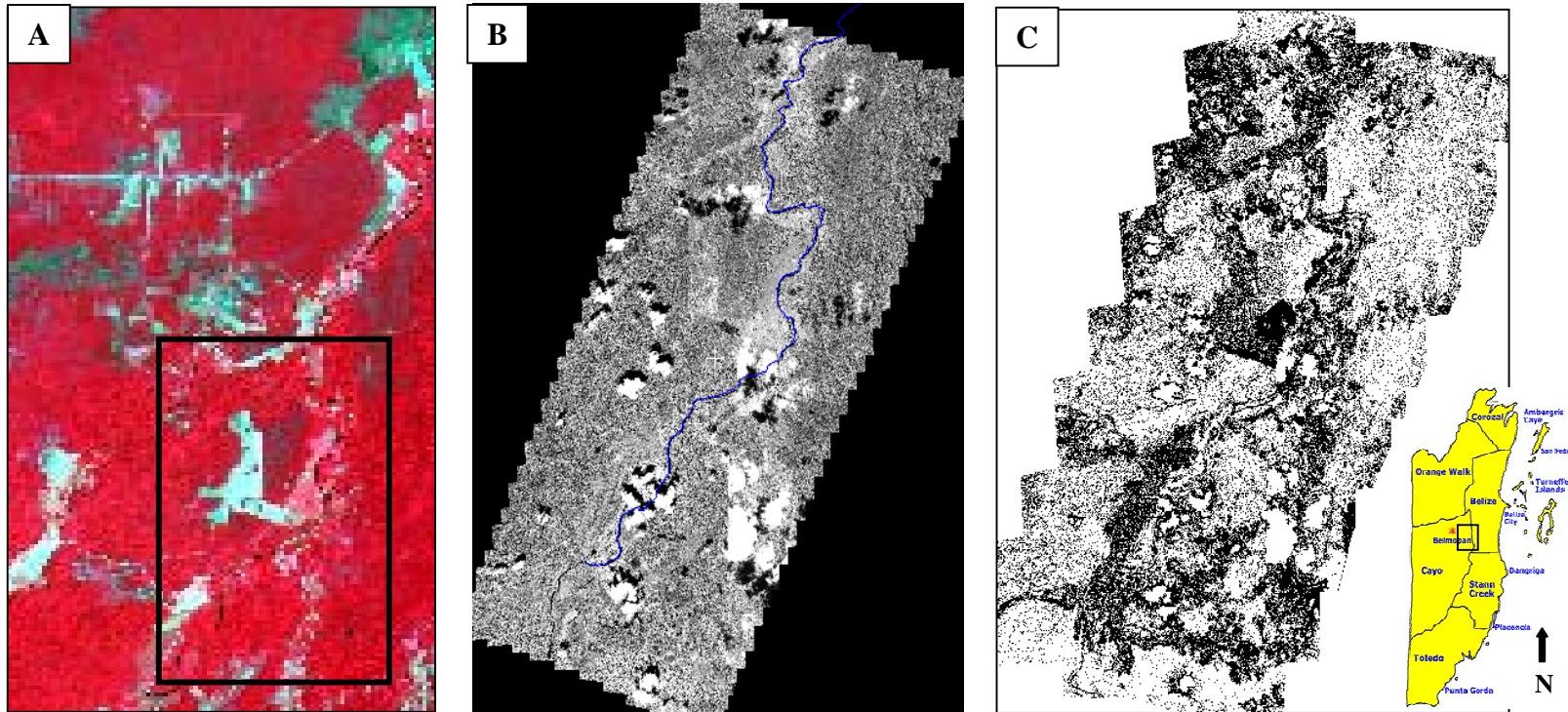


Figure 6. (A) A subset of a SPOT 1998 multispectral image was extracted to include only the (B) IKONOS 2002 scene of the Sibun River study site. Land cover categories of forest, orchard, pasture and gravel/ bare ground generated by an unsupervised classification method were then compared using remote sensing software. (C) A new image was generated to illustrate areas that agree (white) and disagree (black).

Table 1. Feature attributes of both positive and negative detritus mats surveyed on the Sibun River in September 2002.

Attribute	<i>An. darlingi</i> n=162		<i>An. albimanus</i> n=49		<i>An. pseudopunctipennis</i> n=5	
	Positive	Negative	Positive	Negative	Positive	Negative
Habitat #	36	18	19	35	2	52
Area (m)	3.2	5.3	3.6	4.1	2.5	3.9
Shade ¹	28/36	18/18	16/19	29/35	2/2	44/52
% Sticks	72.2	72.4	70.3	76.4	80.0	74.0
% Leaves	19.3	16.8	25.4	18.9	30.0	20.8
% Seeds	2.7	7.1	2.4	6.1	12.5	4.5
% Flowers	1.8	1.4	1.6	3.3	0.0	2.7
% Trash ²	0.6	0.7	0.3	0.8	0.0	0.7
% Foam	3.7	2.1	5.3	4.1	0.0	4.7

¹Characterized as being present at any time during daylight hours.

²Glass and plastic bottles, bags and diapers etc.

Table 2. Results from a habitat survey within a 48-km transect of the Sibun River in September 2002. Although anophelines were the target group, *Chagasia bathana* were also captured.

Species	Life Stage					Total
	1 st instar	2 nd instar	3 rd instar	4 th instar	Pupae	
<i>An. darlingi</i>	45	45	37	34	1	162
<i>An. albimanus</i>	18	14	14	1	2	49
<i>An. pseudopunctipennis</i>	2	1	2	0	0	5
<i>Chagasia bathana</i>	0	1	0	1	0	2
Total	65	61	53	36	3	218

Table 3. Descriptive table of aquatic invertebrates sampled from detritus mats during a habitat survey of *An. darlingi* in the Sibun River conducted in September 2002.

Habitat	Order	Family	Genus
<i>An. darlingi</i> +	Coleoptera	Carabidae	- (1)
		Dytiscidae	<i>Laccophilus</i> (6) <i>Neobidessus</i> (1)
		Gyrinidae	<i>Gyretes</i> (1)
		Hydraenidae	<i>Hydraena</i> (1)
		Hydrochidae	<i>Hydrochus</i> (2)
		Scirtidae	<i>Scirtes</i> (18)
	Collembola	Sminthuridae	<i>Sminthurus</i> (1)
	Diptera	Ceratopogonidae	<i>Bezzia</i> (4) <i>Alluaudomyia</i> (1)
		Chaoboridae	<i>Chaoborus</i> (1)
		Chironomidae	<i>Corynoneura</i> (1) <i>Paramerina</i> (1) <i>Polypedilum</i> (7)
	Ephemeroptera	Baetidae	<i>Baetis</i> (4)
		Leptophlebiidae	<i>Farrodes</i> (1) <i>Leptophlebia</i> (3)
	Hemiptera	Gerridae	<i>Rheumatobates</i> (1)
		Notonectidae	<i>Notonecta</i> (1) <i>Buenoa</i> (5)
		Naucoridae	<i>Ambrysus</i> (3)
		Pleidae	- (1)
		Veliidae	<i>Microvelia</i> (7) <i>Platyvelia</i> (1) <i>Rhagovelia</i> (15) <i>Steinovelia</i> (1)
	Odonata	Libellulidae	<i>Pachydiplax</i> (1)
		Protoneuridae	<i>Protoneura</i> (1)
	Trichoptera	Leptoceridae	<i>Nectopsyche</i> (1)
<i>An. darlingi</i> (-)	Coleoptera	Scirtidae	<i>Scirtes</i> (7)
	Diptera	Ceratopogonidae	<i>Bezzia</i> (1)
		Chironomidae	<i>Polypedilum</i> (1)
	Ephemeroptera	Baetidae	<i>Acerpenna</i> (1) <i>Baetis</i> (1)
		Leptophlebiidae	<i>Leptophlebia</i> (2)
	Hemiptera	Notonectidae	<i>Buenoa</i> (2)
		Veliidae	<i>Microvelia</i> (6) <i>Platyvelia</i> (1)
	Lepidoptera	Pyralidae	- (1)
	Odonata	Protoneuridae	<i>Protoneura</i> (2)
	Trichoptera	Hydropsychidae	<i>Leptonema</i> (1)

Table 4. The contribution of landscape features to both positive and negative *An. darlingi* lodged habitats identified during a survey of the Sibun River in September 2002.

Landscape Feature	Habitats Lodged (n=54)	Contribution of Individual Feature			Combined Components	
		Total %	Positive Habitats (n=36)	Negative Habitats (n=18)	Positive Habitats (n=36)	Negative Habitats (n=18)
Fallen Tree	28	51.9%	21 (58.3%)	7 (38.9%)	27 (75.0%)	10 (55.5%)
Fallen Branch	6	11.1%	4 (11.1%)	2 (11.1%)		
Root System	3	6.0%	2 (5.56%)	1 (5.55%)		
Dried Fallen Bamboo	8	15.0%	5 (13.9%)	3 (16.7%)	6 (16.7%)	3 (16.7%)
Overhanging Live Bamboo	1	2.0%	1 (2.78%)	0		
Overhanging Vegetation ¹	4	7.0%	2 (5.56%)	2 (11.1%)	3 (8.33%)	3 (16.7%)
Vine	2	4.0%	1 (2.78%)	1 (5.56%)		
Eddy	1	2.0%	0	1 (5.56%)	-	-
Other ²	1	2.0%	0	1 (5.56%)	-	-

¹Overhanging vegetation other than bamboo.

²A single stick bridging two semi-submersed rocks.

Table 5. Distances of both positive and negative *An. darlingi* habitats to houses within a 1,000-m search radius identified through photointerpretation of an IKONOS satellite image within the Sibun River study site. Habitats were mapped during a survey conducted to analyze associations between landscape features and detritus mat formations.

Descriptions	Habitat #	House #	Distance (m)
<i>An. darlingi</i> + Homes: 8 Habitats: 13 Avg. House/Habitat: 1.8 Avg. Distance: 546.32 m	2 (R)	28 29 30	757.96 832.14 198.37
	3 (R)	27	563.28
	1 (L)	2	631.64
	2 (L)	2	631.29
	3 (L)	22	553.08
		23	203.32
	4 (L)	22	519.78
		23	274.76
		24	948.84
	5 (L)	22	519.63
		23	283.15
		24	940.99
	7 (L)	27	89.33
	8 (L)	27	507.08
	9 (L)	27	635.15
	10 (L)	27	647.59
<i>An. darlingi</i> – Homes: 9 Habitats: 9 Avg. House/Habitat: 1.4 Avg. Distance: 708.49 m	12 (L)	28	522.11
		29	597.68
		30	564.12
	13 (L)	28	524.25
		29	599.78
		30	566.26
	1 (L)	1	313.40
	2 (L)	16	786.26
	3 (L)	16	779.54
	4 (L)	27	499.94
	1 (L)	1	609.26
		2	890.73
	2 (R)	2	811.58
	3 (R)	2	807.53
	5 (R)	22	776.14
		23	146.00
	6 (R)	28	892.17
		29	965.72
		30	932.11

Table 6. The contribution of different river characteristics to the total pixel count within either a 10 m circular or 4 m polygon buffer zone surrounding both positive and negative *An. darlingi* habitats. Additional analyses were performed to quantify the percentage of river characteristics surrounding habitats formed by fallen trees or other landscape features (i.e., overhanging vegetation, eddy).

Buffer Zone	Description	Deep (>3 m)	Semi-Deep (1-3 m)	Shallow (<1 m)	Riffle	Sandbar	Total Count
10 m circular	+ Habitat n=19	9.80% (422)	61.9% (2,646)	20.4% (874)	7.10% (306)	0.52% (22)	4,270
	- Habitat n=9	45.5% (966)	27.1% (575)	8.20% (174)	20.3% (432)	2.54% (54)	2,121
	Tree n=16	21.6% (754)	48.3% (1,685)	19.9% (697)	8.37% (292)	1.72% (60)	3,488
	No Tree n=12	19.1% (554)	52.9% (1,536)	12.1% (351)	15.3% (446)	0.55% (16)	2,903
4 m polygon	+ Habitat n=19	7.87% (24)	65.9% (201)	15.7% (48)	10.5% (32)	0	305
	- Habitat n=9	40.6% (78)	34.4% (66)	11.4% (22)	13.5% (26)	0	192
	Tree n=16	25.0% (65)	56.2% (146)	12.7% (33)	6.15% (16)	0	260
	No Tree n=12	15.6% (37)	51.1% (121)	15.6% (37)	17.7% (42)	0	237

Table 7. Surface water flow rates sampled at 14 locations within the Sibun River study site in September 2003. Flow rates were determined by measuring the time required for a 1-in. thick foam disc of 3-in. diameter to float along a 1-m transect in three trials from both riverbanks (Left=L; Right=R) and a center site (C) at each location. A negative flow rate indicates an upstream direction.

Location	Site	Water Depth (cm)	Trial	Flow rate (sec)	River Width (m)	River Description
1	L	25	1	> -1 MIN	32	Adjacent to pasture on left riverbank. Permanent Water Usually pond-like with very little current except during high rains.
			2	> -1 MIN		
			3	> -1 MIN		
	C	75	1	7.5		
			2	9.4		
			3	6.8		
	R	25	1	12.3		
			2	12.5		
			3	12.1		
2	L	21	1	4.81	32	Adjacent to pasture on left riverbank. Permanent Water Riffle area 25 m downstream of location 1.
			2	4.98		
			3	4.47		
	C	26	1	2.33		
			2	2.35		
			3	2.43		
	R	12	1	1.85		
			2	1.69		
			3	1.71		
3	L	38.5	1	> -1 MIN	21	Adjacent to pasture on left riverbank. Permanent Water Adjacent to bamboo downstream of location 1 and 2.
			2	0 FLOW		
			3	> -1 MIN		
	C	91	1	4.33		
			2	4.48		
			3	3.72		
	R	47	1	> 1 MIN		
			2	> 1 MIN		
			3	46		
4	L	18	1	7.32	34	Downstream from 1,2 and 3. Permanent Water Immediately before a riffle area and sandbar island.
			2	7.15		
			3	7.66		
	C	49	1	6.57		
			2	7.02		
			3	8.52		
	R	26	1	25.9		
			2	37.9		
			3	30.4		

Location	Site	Water Depth (cm)	TRIAL	Flow rate (sec)	River Width (m)	River Description
5	L	20.5	1	6.6	18	Upstream from 1. Permanent Water
			2	6.5		
			3	7.2		
	C	56	1	1.95		
			2	1.74		
			3	1.99		
	R	28	1	2.53		Usually always stationary current except for periods of high rain when current is stronger.
			2	2.43		
			3	2.55		
6	L	25	1	3.79	28	Sandbar area at end of Sibun River transect.
			2	3.7		
			3	3.86		
	C	80	1	2.25		Permanent Water
			2	2.24		
			3	2.79		
	R	60	1	8.24		Very fast moving water but it was slower than the year of the study.
			2	6.54		
			3	6.98		
7	L	50	1	8.69	33	Upstream from 6 about 30 meters.
			2	8.48		
			3	8.42		
	C	95	1	4.1		Permanent Water
			2	4.08		
			3	3.69		
	R	85	1	15.55		
			2	15.45		
			3	11.83		
8	L	30	1	2.88	33	Upstream from 7 about 30 meters.
			2	3.12		
			3	3.01		
	C	85	1	2.49		Permanent Water
			2	2.72		
			3	2.28		
	R	55	1	5.48		Extremely deep area about 30 meters upstream.
			2	5.51		
			3	5.04		
9	L	30	1	6.84	58	Downstream from the Sibun River bridge about 500 meters.
			2	6.18		
			3	6.16		
	C	90	1	7.11		Semi-permanent Water
			2	7.17		
			3	10.95		
	R	25	1	> 1 MIN		River was more shallow than when first floated. Heavy equipment dredged a channel.
			2	> 1 MIN		
			3	> 1 MIN		

Location	Site	Water Depth (cm)	TRIAL	Flow rate (sec)	River Width (m)	River Description
10	L	15	1	3.79	30	Downstream from 9 about 50 meters.
			2	3.63		
			3	3.56		
	C	30	1	3.93		Semi-permanent Water Before a riffle.
			2	4.27		
			3	3.99		
	R	30	1	9.22		
			2	8.25		
			3	7.93		
11	L	25	1	6.72	37	Downstream from 10 about 2 km.
			2	7		
			3	6.89		
	C	100	1	5.68		Permanent Water Upstream from the head of a creek. Always a current.
			2	6.5		
			3	6.83		
	R	26	1	7.23		
			2	8.42		
			3	7.97		
12	L	130	1	> 1 MIN	55	Upstream from 11. Permanent Water
			2	> 1 MIN		
			3	> 1 MIN		
	C	90	1	> 1 MIN		Orange orchard on left riverbank. Always deep and current is consistent, not fast except when flooded.
			2	> 1 MIN		
			3	> 1 MIN		
	R	30	1	-21.1		
			2	-18.74		
			3	-21.94		
13	L	25	1	13.97	45	Downstream from 12 about 1 km. Permanent Water
			2	11.61		
			3	13.92		
	C	90	1	9.07		Adjacent to pasture on left. River deep upstream with little current.
			2	8.59		
			3	8.32		
	R	112.5	1	15.89		
			2	13.5		
			3	17.13		
14	L	10	1	3.27	24	Downstream from 13 about 50 meters. Permanent Water
			2	2.91		
			3	3.26		
	C	40	1	1.48		Adjacent to pasture on left at riffle area.
			2	1.83		
			3	1.79		
	R	12.5	1	3.07		
			2	3.08		
			3	2.87		

Table 8. Results of an unsupervised classification of the 2002 IKONOS Sibun River scene showing the total percentage of individual land cover categories encompassed within a 20 m buffer zone generated around both positive and negative *An. darlingi* habitats. Additional analyses were performed to quantify the percentage of land cover surrounding habitats formed by fallen trees or other landscape features (i.e., overhanging vegetation, eddy).

Habitat Description	Land Cover Category ¹				Total Count
	Forest	Orchard	Pasture	BareGround/Gravel	
+ Habitat n=19	4.47% (773)	80.2% (13,853)	13.3% (2,297)	2.04% (353)	17,276
- Habitat n=9	5.05% (444)	80.5% (7,072)	13.3% (1,164)	1.18% (104)	8,784
Tree n=16	5.47% (755)	79.7% (11,012)	12.4% (1,711)	2.41% (333)	13,811
No Tree n=12	3.77% (462)	80.9% (9,913)	14.3% (1,750)	1.01% (124)	12,249

¹ Land cover was determined by a 20-iteration 30-class isodata unsupervised classification then further grouped into four general land cover categories using photointerpretation based on field land cover data.

Table 9. Confusion matrix of land cover within the Sibun River study site determined by reference points of an IKONOS 2002 image and that determined by a SPOT 1998 subset of the same area after performing unsupervised classifications. A total of 36 random sampling points were used to compare land cover between the images. Each value represents the number of counts for a particular pair of classes.

SPOT 1998 Data	IKONOS 2002 Data ¹				Total Counts
	Forest	Orchard	Pasture	Gravel	
Forest	19	6	5	2	32
Orchard	0	1	0	0	1
Pasture	0	0	1	1	2
Gravel	1	0	0	0	1
Total	20	7	6	3	36
% Agreement	95.0%	14.3%	16.7%	0%	

¹Land cover was determined by a 20-iteration 30-class isodata unsupervised classification then further grouped into four general land cover categories using photointerpretation based on field land cover data.

Chapter 7

General Conclusion

GENERAL CONCLUSION

Despite decades of interest, research and organized control efforts, malaria continues to be a major health threat for those living in endemic countries. An excess of 2.1 billion people are estimated to live in such regions (WHO 1991). Unfortunately, malaria transmission has increased in recent years due in part to a lack of resources, a weakened support for the use of effective insecticides, parasite drug resistance and the continuing movement of infected and/or susceptible human populations throughout endemic areas. In addition, population expansion and the resulting economic demands have led to an increase in land use changes, including deforestation, due to human settlement and agricultural growth. Such landscape changes may promote anopheline vector breeding habitats and potentially increase disease transmission (Walsh et al. 1993; Patz et al. 2000; Conn et al. 2002). Alternatively, landscape changes can have a negative impact on vectors and decrease potential for disease transmission (WHO 1982). For these reasons, it is vital that vector bionomic studies continue to be supported in endemic countries in order to provide sustainable solutions for mosquito management and control.

The ultimate goal of vector research is to decrease human-vector contact. This includes the reduction of breeding sites, the interruption of adult feeding and the placement of homes in low-risk areas. However, detailed knowledge of local anopheline adult and larval ecology must first be acquired before efficacious tools to predict high-risk transmission areas can be developed and proper control measures can be implemented. An interdisciplinary effort can provide this required information. For example, field studies can be used to determine adult characteristics that define a species' vectorial capacity including peak biting times, seasonal population densities and flight

behaviors. In addition, larval ecology research can be performed to define those environmental parameters that are required for preferred breeding habitats. These data can then be combined with remote sensing and geographical information system (GIS) technologies to predict potential high-risk areas of malaria transmission through spatial analyses (Andre et al. 1995). Conclusions from this kind of research can then be used to guide decision-making processes regarding disease surveillance and resource allocation within malaria control programs.

Such studies have been conducted in Central and Latin America, but one important anopheline that has had limited attention in this region is *Anopheles darlingi* Root. This species is considered the most efficient malaria vector in the New World (Foote and Cook 1959) and, where it occurs, has been found to be the major or only vector of human malaria in South America (Forattini 1962; Lourenco-de-Oliveira et al. 1989). Despite its widespread distribution and the substantial vectorial capacity of this anopheline as a malaria transmitter in other countries, only a small amount of research has focused on *An. darlingi* in the country of Belize since its first discovery in 1940 (Komp 1940; Kumm and Ram 1941; Manguin et al. 1996; Harbach et al. 1993; Roberts et al. 1993; Rejmankova et al. 2000; Grieco 2001; Achee et al. 2000; Roberts et al. 2002). For this reason, it was important to further describe the natural history of this vector and to define tools that could be used to predict high-risk areas for *An. darlingi* populations.

There are several key behavioral characteristics that have been described in the present research that indicate the importance of *An. darlingi* in malaria transmission. The current study is the first to report on the late-night biting pattern of adults. We found that *An. darlingi* is an endophagic species, entering homes to feed, and will bite in relatively

high numbers indoors throughout the night. Such behavior signifies a strong relationship with humans because host-choice is reduced inside houses during nocturnal hours. This is further supported by results generated in the flight behavior study that showed marked females will return to a house located 800 M from a release point to feed on human bait. This refers to an approximate 2-km² area in which to seek a host. Such anthropophilic behavior indicates an increased probability of human-vector contact and therefore is extremely important in determining the risk of malaria transmission at various proximities from larval habitats. In addition, results from monitoring the seasonal trend of population densities illustrated peaks in *An. darlingi* population densities prior to an increase in reported malaria cases. However in order to truly define the role of *An. darlingi* in local disease incidence, microepidemiological studies combining active case detection with systematic vector surveys are required and should be pursued in the future.

The flight behavior of *An. darlingi* in Belize was unknown prior to the current research. In addition, the study design, incorporating the use of one fixed release site and a portable experimental hut, is a novel approach. Because this study was the first to gather baseline data on the flight behavior of *An. darlingi* in Belize, this design was chosen in order to simplify data interpretation. In addition, using a fixed release point reduced the potential for variation in environmental factors (i.e., wind direction, landscape features, temperature, sun exposure, etc.) that may have occurred if using multiple release sites and eliminated one possible confounding variable when analyzing the recapture data. Future studies should now manipulate the parameters of both the release and recapture sites to examine more detailed flight behavior patterns.

The success of both the study and hut designs have provided the groundwork to conduct flight studies of two other anophelines, *An. albimanus* and *An. vestitipennis*, in the northern region of Belize. Results from that research indicate a significantly reduced return rate at the 0 M distance for both species compared to *An. darlingi*, and no recaptures of *An. albimanus* were made at the 400 M or 800 M site (Grieco pers. comm.). In addition, no *An. vestitipennis* females were recaptured at the 800 M distance. This information is extremely important in spatial analyses used to define high-risk areas for human-vector contact and to compare these risks between anopheline species.

Being a riparian mosquito, *An. darlingi* is associated with river habitats throughout its geographic distribution (Faran and Linthicum 1981). Within Belize specifically, Manguin et al. (1996) described the ecological determinants of larval habitats to include floating mats of debris in shaded areas along freshwater river margins. In particular, the floating mats were a result of overhanging bamboo and submersed roots along the riverbank. Because the spatial distribution of malaria transmission is influenced by the location of vector breeding sites, it was important to describe the function of bamboo in both *An. darlingi* habitat selection and formation. In addition, deforestation occurring along river systems known to harbor *An. darlingi* habitats created the urgency for detailed information regarding the association between land cover and the presence of bamboo. Data from these studies could then be used to suggest management options.

Using floating enclosure traps, overhanging bamboo was determined not to be used as a selection criterion for habitat preference but instead its role was suggested to lodge detritus mats that are then used for egg deposition and/or to accumulate debris containing *An. darlingi* larvae. In addition, a systematic survey of the Sibun River

described the total contribution of bamboo to debris patch lodging to be less than the contribution by tree components. Unfortunately, because of the transitory nature of rivers and debris material, the management of these fallen trees to reduce habitat formation would require substantial financial and logistical support and may not be feasible.

The use of floating enclosure traps to determine the role of bamboo in habitat selection ensured the stability of detritus mat treatments (i.e., debris could not float downstream). This allowed for several sampling replications. If natural habitat surveys were to have been employed, detritus mats would have been vulnerable to degradation due to quick changes in river current and may not have been available for repeated sampling. In addition, due to the limited number of natural habitats matching the treatments under investigation in the present study, it would have been difficult to standardize extraneous factors (i.e., flow rate, sun exposure, water temperature and depth, detritus composition and mat size, overhanging bamboo density, etc.). This was not a problem using the floating containers because the same treatments were used in replicate and all traps were placed in close proximity to each other within the same study site thereby reducing environmental variability.

Results from combined larval ecology and remote sensing studies indicated that the ability to predict locations of *An. darlingi* breeding habitats based on the direct observation of bamboo growth in satellite images or through land cover surrogates along river margins cannot be supported. In addition, characteristics of the river system (i.e., water depth, location of sandbars and riffles) also proved to be unhelpful in determining the location of potential *An. darlingi* larval sites. Although the distance of mapped debris patches to surrounding homes within a 1,000-m search radius was significantly less for

positive mats, the suggestion to target *An. darlingi* larval control efforts based on house location cannot be made. The location of many homes along river systems is unstable, especially in regards to immigrants, and accessibility to these structures can be difficult; therefore, high-resolution satellite imagery would have to be utilized and updated on a yearly basis in order to establish current house sites. In addition, because of the reduced scene size of such images, several scenes would have to be purchased in order to encompass entire riverine systems. For these reasons, it would not be cost-effective for the Belize malaria control program to use remote sensing and GIS tools for the specific purpose of targeting *An. darlingi* breeding sites.

The suggestion that people build their homes further away from rivers to reduce *An. darlingi*/human contact also cannot be made. The flight behavior study in the present research did not access maximum flight distance. It is known that *An. darlingi* has the ability to fly over many kilometers (Charlwood and Alecrim 1989) and placing homes away from rivers based on these distances would not be practical for people who require access to river systems to fish, gather drinking water or for bathing. Most importantly, even though the rate of recapture reduced as the portable hut was moved further away from the release site, natural biting populations remained high at all distances. *Anopheles darlingi* breeds in small creeks and streams in addition to rivers, and these habitats will be scattered throughout riverine systems. The identification of all available breeding habitats would have to be identified, and setting one “safe” distance that a house would have to be placed from every location would be extremely difficult.

Based on data gathered in the present research, the characteristics and availability of preferred *An. darlingi* breeding habitats deem control measures targeting larval

populations neither cost-effective nor practical. The most productive means for reducing the risk of *An. darlingi*/human contact appears to be control efforts targeting adults, including routine residual house spraying. Even though continued research efforts are necessary to fully characterize the role of *An. darlingi* in disease transmission within Belize, data presented in this document have added to our current understanding of the importance of this vector. This information will provide public health officials the ability to better understand the role of *An. darlingi* in malaria transmission within the central region of the country and guide decision-making processes regarding the incorporation of remote sensing and GIS technologies to predict specific locations within rivers at high-risk for *An. darlingi* larval habitat formation.

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APPENDIX I

Anopheline Collections During Preliminary Study Site Search

APPENDIX I

VILLAGE	DISTRICT	DATE	<i>An. darlingi</i>	<i>An. albimanus</i>	<i>An. crucians</i>	<i>An. punctimacula</i>	<i>An. pseudopunctipennis</i>	<i>An. vestitipennis</i>
Guinea Grass	Orange Walk	7/5/01	0	22	1	0	0	20
Carmelita	Orange Walk	7/6/01	0	12	0	0	0	12
Douglas	Orange Walk	7/9/01	1	65	1	0	0	9
		7/20/01	2	112	1	6	0	31
Tower Hill	Orange Walk	7/10/01	0	23	0	0	0	2
San Roman	Orange Walk	7/15/01	4	229	4	0	0	22
		7/19/01	5	241	1	1	0	5
Bladen Reserve	Stann Creek	7/19/01	4	0	0	2	0	0
Maskall	Belize	7/24/01	10	35	0	0	0	2
San Estevan	Orange Walk	8/9/01	0	71	0	2	0	30
Sibun	Cayo	8/11/01	35	3	0	5	3	0
		9/5/01	88	20	0	4	0	0
Orange Walk	Orange Walk	7/23/01	0	33	0	2	0	20
		9/17/01	3	170	1	0	0	10

Appendix I. Anopheline species captured from 6:30-8:00 pm in human-baited landing collections from various villages in Belize, Central America during a preliminary search for a study site.

APPENDIX II

Armenia House Survey

APPENDIX II

Appendix II. Data from a house survey performed in the village of Armenia located in the central Cayo District of Belize, Central America. Armenia was chosen because it is the closest village to the study site. This survey was used to determine comparability of the experimental hut to indigenous homes based on type of construction material, the numbers of windows and doors and area of living space. W=Sleeping and living quarters, S=Sleeping quarters only.

House #	Roof	Walls	Floor	Doors	Windows	Width (FT)	Length (FT)	Descriptor	Area (FT)
1	ZINC	WOOD	CEMENT	1	3	9	18	S	162
2	THATCH	WOOD	GROUND	2	3	15	23	W	345
3	THATCH	WOOD	GROUND	1	2	9	15	W	135
4	OIL ZINC	WOOD	GROUND	2	2	18	28	W	504
5	THATCH	WOOD	GROUND	1	3	13	16	W	208
6	THATCH	WOOD	GROUND	2	0	15	35	W	525
6	ZINC	WOOD	CEMENT	2	2	13	24	W	312
7	THATCH	WOOD	GROUND	2	0	28	20	W	560
8	THATCH	WOOD	GROUND	1	0	13	21	S	273
9	THATCH	WOOD	GROUND	2	0	13	29	W	377
10	THATCH	WOOD	GROUND	2	0	12	28	W	336
11	THATCH	WOOD	GROUND	2	0	20	21	W	420
12	THATCH	WOOD	GROUND	2	0	20	20	W	400
13	ZINC	WOOD	GROUND	1	2	10	21	W	210
14	THATCH	WOOD	GROUND	2	0	12	25	W	300
15	ZINC	WOOD	CEMENT	2	4	18	21	S	378
16	THATCH	WOOD	GROUND			21	19	S	399
17	THATCH	WOOD	CEMENT	2	1	16	18	S	288
18	THATCH	NONE	CEMENT	2	5	17	21	W	357
19	THATCH	WOOD	GROUND	1	1	15	18	S	270
20	ZINC	WOOD	CEMENT	2	3	16	21	S	336
21	THATCH	WOOD	GROUND	2	0	18	21	W	378
22	OIL ZINC	WOOD	GROUND	1	3	18	15	S	270
23	THATCH	WOOD	GROUND	1	0	17	14	S	238
24	ZINC	WOOD	CEMENT	2	2	24	17	S	408
25	THATCH	WOOD	CEMENT	1	2	9	20	S	180
26	THATCH	WOOD	CEMENT	1	0	12	17	W	204
27	ZINC	WOOD	CEMENT	1	2	14	20	W	280
28	ZINC	CEMENT	CEMENT	2	2	17	10	S	170
29	ZINC	WOOD	WOOD	2	8	18	18	W	324
30	ZINC	WOOD	CEMENT	2	2	17	16	W	272
31	OIL ZINC	WOOD	CEMENT	1	2	18	24	W	432
32	ZINC	NONE	CEMENT	2	8	22	31	W	682
33	ZINC	WOOD	WOOD	1	1	11	18	S	198
34	ZINC	WOOD	CEMENT	1	2	11	20	S	220
35	ZINC	WOOD	GROUND	1	1	9	21	S	189
36	THATCH	WOOD	GROUND	2	0	14	21	W	294
37	ZINC	WOOD	GROUND	2	0	9	23	S	207

House #	Roof	Walls	Floor	Doors	Windows	Width (FT)	Length (FT)	Descriptor	Area (FT)
38	ZINC	WOOD	CEMENT	2	5	15	30	W	450
39	ZINC	WOOD	CEMENT	1	4	16	12	W	192
40	THATCH	WOOD	GROUND	1	1	12	18	W	216
41	ZINC	WOOD	CEMENT	2	2	16	25	S	400
42	THATCH	WOOD	CEMENT	2	4	21	20	W	420
43	ZINC	WOOD	CEMENT	2	5	18	23	W	414
44	ZINC	WOOD	WOOD	2	8	18	24	W	432
45	ZINC	WOOD	WOOD	2	6	21	26	W	546
46	ZINC	NONE	CEMENT	3	3	22	26	S	572
47	ZINC	WOOD	CEMENT	2	4	15	20	S	300
48	ZINC	WOOD	WOOD	2	4	15	22	W	330
49	ZINC	NONE	CEMENT	2	4	16	20	S	320
50	OIL ZINC	WOOD	GROUND	2	1	15	17	S	255
51	ZINC	NONE	CEMENT	3	5	20	33	S	660
52	OIL ZINC	WOOD	GROUND	1	2	10	17	S	170
53	ZINC	WOOD	CEMENT	1	3	9	16	S	144
54	ZINC	WOOD	GROUND	2	0	15	21	S	315
55	ZINC	WOOD	CEMENT	2	1	19	16	S	304
56	ZINC	WOOD	CEMENT	2	1	11	15	S	165
57	OIL ZINC	WOOD	GROUND	2	1	15	16	S	240
58	ZINC	CEMENT	CEMENT	2	6	23	36	W	828
59	ZINC	CEMENT	CEMENT	2	3	18	24	W	432
60	ZINC	CEMENT	CEMENT	3	4	21	27	W	567
61	OIL ZINC	WOOD	GROUND	1	1	19	23	W	437
62	ZINC	WOOD	CEMENT	2	3	13	22	S	286
63	THATCH	WOOD	CEMENT	2	0	14	20	S	280
64	ZINC	NONE	GROUND	0	0	13	12	W	156
65	ZINC	NONE	CEMENT	2	4	19	26	W	494
67	ZINC	WOOD	CEMENT	2	4	16	19	W	304
68	ZINC	NONE	CEMENT	2	3	22	23	W	506
69	ZINC	WOOD	CEMENT	2	2	12	16	S	192
70	ZINC	CEMENT	CEMENT	2	7	18	24	W	432
71	ZINC	CEMENT	CEMENT	4	2	24	20	W	480
72	THATCH	WOOD	GROUND	2	0	20	28	S	560
73	ZINC	WOOD	CEMENT	2	0	19	22	S	418
74	ZINC	WOOD	GROUND	3	1	20	24	S	480
75	THATCH	WOOD	CEMENT	2	3	17	13	S	221
76	THATCH	WOOD	GROUND	2	2	19	20	W	380
77	ZINC	WOOD	CEMENT	3	2	24	18	W	432
78	THATCH	WOOD	GROUND	1	0	13	17	W	221
79	ZINC	WOOD	GROUND	2	0	17	11	W	187
80	ZINC	CEMENT	CEMENT	2	4	17	20	S	340
81	THATCH	WOOD	CEMENT	1	3	15	12	S	180
82	THATCH	WOOD	GROUND	2	0	10	12	W	120
83	THATCH	WOOD	GROUND	2	0	13	24	S	312

House #	Roof	Walls	Floor	Doors	Windows	Width (FT)	Length (FT)	Descriptor	Area (FT)
84	ZINC	WOOD	WOOD	1	4	16	19	S	304
85	THATCH	WOOD	GROUND	1	0	12	19	W	228
86	THATCH	WOOD	GROUND	2	0	14	29	W	406
87	THATCH	WOOD	GROUND	1	0	13	30	W	390
88	ZINC	WOOD	CEMENT	2	5	15	24	W	360
89	THATCH	WOOD	GROUND	2	0	12	19	W	228
90	ZINC	WOOD	CEMENT	1	0	13	23	S	299
91	THATCH	WOOD	GROUND	1	0	14	23	W	322
92	THATCH	WOOD	GROUND	2	0	12	20	W	240
93	ZINC	WOOD	CEMENT	2	2	18	24	W	432
94	ZINC	NONE	GROUND	2	6	16	25	S	400
95	ZINC	WOOD	CEMENT	2	3	12	16	W	192
96	ZINC	WOOD	GROUND	2	5	16	18	W	288
97	ZINC	WOOD	GROUND	2	3	18	22	W	396
98	ZINC	WOOD	GROUND	2	5	16	18	W	288
99	ZINC	WOOD	CEMENT	2	6	12	15	S	180
100	ZINC	WOOD	GROUND	2	3	19	13	S	247

APPENDIX III

Bitting Pattern and Seasonal Adult Collection Form

APPENDIX III

Biting Pattern and Seasonal Adult Collection Form

Date: _____
Collectors:
6-12pm _____
12-6am _____

Location: _____
GPS (UTM/WGS84/M) _____

Hourly Temp/RH:

	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr
RH													
TP													

Hourly Wind:

	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr
SP													
DIR													

Hourly Collections Inside:

Species	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr	Total
DAR														
ALB														
VEST														
PSEUDO														
PUNCT														
Total														

Hourly Collections Outside:

Species	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr	Total
DAR														
ALB														
VEST														
PSEUDO														
PUNCT														
Total														

APPENDIX IV

San Roman Distance Survey

APPENDIX IV

San Roman Village: 78 Houses Mapped

House #	Distance (ft.)	Distance (m)	House #	Distance (ft.)	Distance (m)
1	932	284	115	446	136
4	810	247	116	346	105
5	673	205	117	229	70
6	749	228	118	235	72
7	821	250	119	245	75
8	585	178	120	300	91
13	719	219	121	334	102
16	718	219	122	297	91
17	636	194	123	148	45
27	659	201	124	185	56
50	570	174	125	160	49
52	501	153	126	172	52
53	357	109	127	202	62
54	424	129	127	202	62
55	316	96	128	204	62
56	316	96	129	200	61
57	377	115	130	246	75
58	369	112	131	332	101
59	347	106	132	322	98
60	417	127	133	315	96
61	420	128	134	305	93
62	329	100	135	304	93
63	442	135	136	256	78
64	507	155	138	529	161
65	447	136	139	483	147
66	533	162	140	457	139
67	438	134	141	402	123
68	324	99	142	445	136
69	253	77	143	398	121
70	246	75	144	371	113
71	191	58	145	363	111
72	175	53	146	370	113
73	167	51	147	522	159
75	232	71	11a	653	199
77	146	45			
78	142	43			
105	1071	326			
108	591	180			
109	540	165			
110	518	158			
111	516	157			
112	507	155			
113	546	166			
114	764	233			

Average Distance	414 ft	126 m
Quartiles:		Rounded To:
Lowest	43 meters	50 m
25% Quartile	77 meters	100 m
Median	112 meters	100 m
75% Quartile	155 meters	200 m
Highest	326 meters	400 m

Appendix IV. Results from a survey conducted in the village of San Roman, Orange Walk District, Belize showing the shortest straight-line distances from individual homes to the Rio Hondo River and potential *An. darlingi* breeding sites (See Figure 3, Chapter 3). Distance quartiles were used in flight distance studies.

APPENDIX V

San Estevan Distance Survey

APPENDIX V

Appendix V. Results from a survey conducted in the northern village of San Estevan in the Orange Walk District showing the shortest straight-line distances from individual homes to the New River and potential *An. darlingi* breeding sites. Distance quartiles were used in flight distance studies

San Estevan Village: 210 Houses Mapped					
House #	Distance (ft.)	Distance (m)	House #	Distance (ft.)	Distance (m)
1	1102	336	116	456	139
2	469	143	117	407	124
3	787	240	118	308	94
4	833	254	119	440	134
5	955	291	120	436	133
6	955	291	121	600	183
7	1227	374	122	673	205
8	1089	332	123	686	209
9	1073	327	124	607	185
10	1089	332	125	682	208
11	768	234	126	1175	358
12	768	234	127	1047	319
13	620	189	127	1191	363
14	1135	346	128	958	292
15	1266	386	129	869	265
16	1424	434	130	866	264
17	1345	410	131	883	269
18	1119	341	132	702	214
19	614	187	133	781	238
20	528	161	134	827	252
21	587	179	135	958	292
22	604	184	136	1020	311
23	627	191	138	515	157
24	1309	399	139	669	204
25	495	151	140	653	199
26	748	228	141	1545	471
27	1401	427	142	1811	552
28	1457	444	143	1312	400
29	1562	476	144	1578	481
30	1391	424	145	1742	531
31	1430	436	146	1657	505
32	1627	496	147	1381	421
33	1739	530	148	459	140
34	1657	505	149	453	138
35	1673	510	150	1814	553
36	705	215	151	522	159
37	856	261	152	535	163
38	958	292	153	541	165

House #	Distance (ft.)	Distance (m)	House #	Distance (ft.)	Distance (m)
39	814	248	154	614	187
40	784	239	155	600	183
41	751	229	156	705	215
42	932	284	157	719	219
43	912	278	158	669	204
44	427	130	159	994	303
45	364	111	160	571	174
46	1040	317	161	318	97
47	869	265	162	276	84
48	282	86	163	453	138
49	705	215	164	2303	702
50	427	130	165	2418	737
51	456	139	166	1470	448
52	387	118	167	1585	483
53	325	99	168	1624	495
54	236	72	169	2657	810
55	397	121	170	2569	783
56	492	150	171	1611	491
57	535	163	172	2792	851
58	656	200	173	1804	550
59	538	164	174	935	285
60	725	221	175	942	287
61	518	158	176	217	66
62	732	223	177	299	91
63	774	236	178	584	178
64	889	271	179	787	240
65	843	257	180	830	253
66	1017	310	181	988	301
67	1106	337	182	256	78
68	1037	316	183	659	201
69	1060	323	184	912	278
70	1083	330	185	722	220
71	1122	342	186	912	278
72	1024	312	187	751	229
73	1148	350	188	784	239
74	1142	348	189	846	258
75	1276	389	190	1112	339
76	869	265	191	971	296
77	869	265	192	1086	331
78	699	213	193	696	212
79	676	206	194	755	230
80	630	192	195	709	216
81	538	164	196	1119	341
82	390	119	197	1276	389

House #	Distance (ft.)	Distance (m)	House #	Distance (ft.)	Distance (m)
83	246	75	198	997	304
84	453	138	199	791	241
85	325	99	200	692	211
86	715	218	201	633	193
87	725	221	202	614	187
88	801	244	203	728	222
89	509	155	204	568	173
90	755	230	205	702	214
91	617	188	206	525	160
92	843	257	207	722	220
93	279	85	208	486	148
94	312	95	209	344	105
95	144	44	210	640	195
96	220	67	211	623	190
97	312	95			
98	361	110			
99	344	105			
100	328	100	Average Distance 838 ft 255 m		
101	335	102			
102	384	117	Quartiles:		
103	305	93	Rounded To:		
104	446	136	lowest 41 meters 50 m		
105	535	163	25% quartile 161 meters 160 m		
106	610	186	median 221 meters 200 m		
107	650	198	75% quartile 318 meters 300 m		
108	574	175	highest 851 meters 800 m		
109	535	163			
110	509	155			
111	397	121			
112	135	41			
113	253	77			
114	256	78			
115	1359	414			

APPENDIX VI

Flight Distance Collection Form

APPENDIX VI

Flight Distance Form

Date: _____

Color: Red Blue White Yellow Green

Collectors:

6-12pm _____

12-6am _____

Distance: _____

Release site: _____

GPS (UTM/WGS84/M)

Hourly Temp/RH:

	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr
RH													
TP													

Hourly Wind:

	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr
SP													
DIR													

Hourly Collections Inside:

Species	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr	Total
DAR	C+: C:-													
ALB														
VEST														
PSDO														
PNCT														
Total	C+: C:-													

Hourly Collections Outside:

Species	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	13hr	Total
DAR	C+: C:-													
ALB														
VEST														
PSDO														
PNCT														
Total	C+: C:-													

APPENDIX VII

St. Thomas Larval Survey

APPENDIX VII

St. Thomas Creek (2/25/02)
Enclosure Trap Larval Survey
30 Dips/Habitat - 100 m transect

Species	Habitat				Totals	%
	Grass Margin	Debris	Root Hairs			
<i>Chagasia bathana</i>	2	18	8		28	14.9
<i>Anopheles albimanus</i>	0	2	5		7	3.7
<i>Anopheles darlingi</i>	5	57	16		78	41.5
<i>Anopheles gabaldoni</i>	0	7	12		19	10.1
<i>Anopheles punctimacula</i>	8	11	5		24	12.8
<i>Anopheles vestitipennis</i>	6	19	6		31	16.5
Unknown	1	0	0		1	0.53
Total	22	114	52		188	100%
Percentage of Larvae from All Habitats	11.7%	60.6%	27.6%			
Percentage of <i>An. darlingi</i> /Individual Habitat	22.7%	50.0%	30.7%			

Appendix VII. Results from the larval survey conducted along St. Thomas Creek in the Cayo District of Belize, Central America. All potential anopheline habitats from both sides of the creek along a 100 M transect were sampled to confirm the presence of *An. darlingi* larvae. Data were used in establishing the location for the placement of enclosure traps used in an *An. darlingi* habitat preference study.

APPENDIX VIII

Enclosure Trap Collection Form

APPENDIX VIII

Enclosure Trap Form

	C			D			OB			OB + D		
Trap Set #1	D	A	V/P	D	A	V/P	D	A	V/P	D	A	V/P
Day 5:												
Day 11:												
Day 17:												
Totals:												
Combined Trap Totals:												

	C			D			OB			OB + D		
Trap Set #2	D	A	V/P	D	A	V/P	D	A	V/P	D	A	V/P
Day 5:												
Day 11:												
Day 17:												
Totals:												
Combined Trap Totals:												

	C			D			OB			OB + D		
Trap Set #3	D	A	V/P	D	A	V/P	D	A	V/P	D	A	V/P
Day 5:												
Day 11:												
Day 17:												
Totals:												
Combined Trap Totals:												

	C			D			OB			B + D		
Trap Set #4	D	A	V/P	D	A	V/P	D	A	V/P	D	A	V/P
Day 5:												
Day 11:												
Day 17:												
Totals:												
Combined Trap Totals:												